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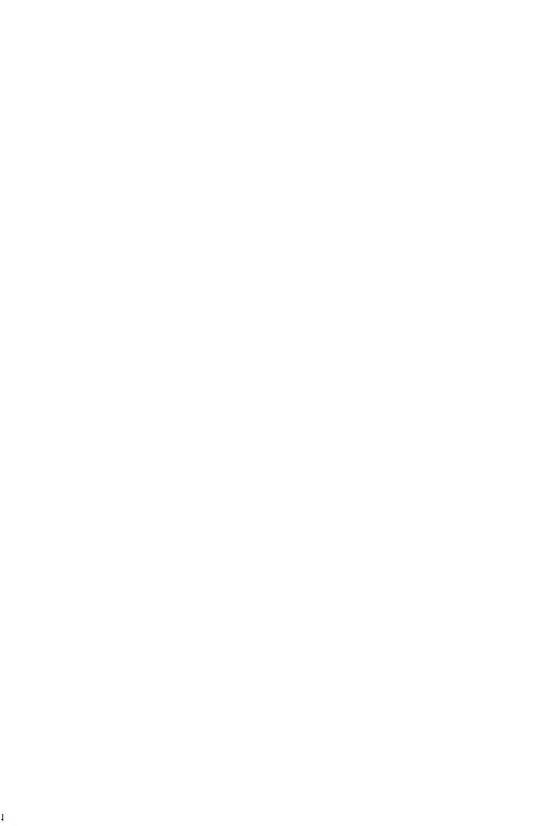
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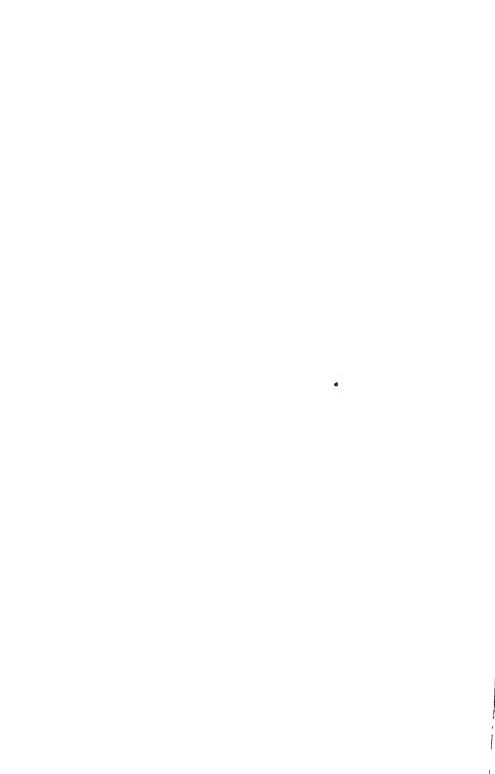
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11337

Movements of Human Diaphragm During Cardiac Cycle in Respiratory Pause.

ANCEL KEYS.

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Diaphragmatic "tug," accompanying ventricular systole in man, has been recognized for many years (cf., e.q., Mackenzie,1). It has not been analyzed but it has been stated that it "can produce but little movement upward because of the inertia of the heavy abdominal organs." (Hamilton.2)

By means of the multiple slit roentgenkymograph of Stumpf3 the component of motion in any given plane of moving boundaries between regions of differing radiographic density can be accurately determined for intervals of time down to about 0.03 second. We

¹ Mackenzie, J., Diseases of the Heart, 1913, 2nd ed., London.

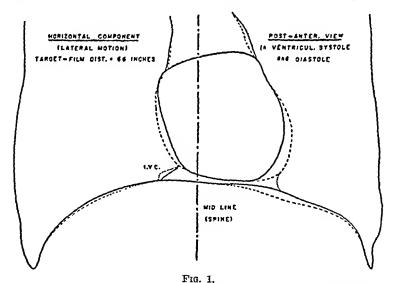
² Hamilton, W. F., Am. J. Physiol., 1930, 91, 712.

³ Stumpf, P., Fortsch. Röntgenstr., 1928, 36, 3.

have used this procedure for measuring the size change and stroke output of the heart during a single cardiac cycle (Keys and Friedell').

Stumpf⁵ noted a cephalad movement of the diaphragm during systole and also stated that this movement may be reversed in the more lateral region of the diaphragm. We have studied a large number of roentgenkymograms (R.K.G.s) to get quantitative measures of these movements as recorded in posterior-anterior, lateral, and oblique views during respiratory pause, in all of which we have made both vertical and horizontal R.K.G.s at a film to target distance of 66 inches. Mathematical analysis of the measurements from tracings on these R.K.G.s was made by regarding the diaphragm as a frustrum of a dome of elliptical section. Rather than assume exact conformity to a simple geometrical form, the displacements recorded were integrated over a large number of steps.

Fig. 1 reproduces the tracing of the horizontal component of motion of both heart and diaphragm in the p.a. view in a typical case (normal young man 5 minutes after moderate exercise). Here, as is generally



Horizontal component of motion of the heart and diaphragm, posterior-anterior view. Exact tracing of position of boundaries at full left ventricular diastole and the next succeeding systole during respiratory pause (moderate inspiration). Normal young man.

⁴ Keys, A., and Friedell, H. L., PROG. Soc. EXP. BIOL. AND MED., 1938, 40, 267; Am. J. Physiol., 1939, 126, 741.

⁵ Stumpf, P., Weber, H. H., and Weltz, G. A., Röntgenkymographische Bewegungslehre innerer Organe, 1936, G. Thieme, Leipzig.

the case, both right and left diaphragms moved laterally in systole and this motion is greatest (maximum 15.5 mm) on the left side near the apex of the heart. The vertical component of motion in the p.a. view in this case showed cephalad motion of the left and central diaphragm, greatest near the apex (maximum 4.5 mm), but with apparent paradoxical motion of most of the right diaphragm (maximum 3 mm). This again is generally the case, as is the appearance of paradoxical motion in the posterior portion of the central and left diaphragm when studied in the lateral R.K.G.

The total net motion of the diaphragm is always such as to reduce the volume of the thorax during systole. Since the 4 films necessary cannot be made simultaneously, exact values cannot be given but in the young man whose R.K.G. tracing is shown here (Fig. 1), the total net volume change of the thorax computed from the p.a. and lateral R.K.G.s was about 30 cc. of which approximately \$0% was the resultant of lateral (horizontal) motion and 20% was pure vertical elevation of the diaphragm. The small value for the net vertical component results from the paradoxical vertical motion of the diaphragm. In this illustrative case the mean stroke output (left ventricle) was about 70 cc from our method of calculation (Keys and Friedell'). The volume reduction of the thorax during the cardiac cycle always corresponds to at least a large fraction of the stroke outpur. These observations on the diaphragm lend further support to our contention that the total heart size in man is markedly reduced in systole and that this reduction is closely related to the stroke output.

We have also studied 8 cases of pneumoperitoneum by these methods. In these cases the viscera are removed from the peritoneum by 5 to 15 cm and not only the free diaphragm but also the whole of the caudal portion of the heart are made sharply visible. The results are in full agreement with our observations on normal individuals where the heart and diaphragm are less fully visualized.

It should be noted that these diaphragmatic and cardiac movements in man are not necessarily identical with those in animals (Hamilton and Rompi") which do not maintain the erect posture and which possess a different architecture about the heart (absence of rigid mediastinum, presence of a frenulum attachment to the apex of the heart, etc.). In man, alteration of posture or of the respiratory phase changes the form of the movement. For example, the movements of the diaphragm are more difficult to visualize in expiration and they tend to disappear in the Valsalva experiment. In all cases the total thoracic volume tends to diminish in systole by an amount

⁶ Hamilton, W. F., and Rompi, J. H., Am. J. Physick, 1902, 102, 559.

which appears to correspond to a large fraction of the systolic discharge. During respiration these passive movements are obscured by the active movements of the diaphragm but if the stroke volume is large and respiration is very quiet traces may still be visible in the R.K.G.

Summary. In man during respiratory pause there are changes in the position of the diaphragm during the cardiac cycle so that in ventricular systole the total thoracic volume is smaller than in diastole. Calculation of the net thoracic changes involved integration of measurements of both vertical and horizontal components of motion in posterior-anterior and lateral projections. The reduction in thoracic volume amounts to a large and apparently rather constant fraction of the cardiac stroke output.

11338

Production of Bradycardia in Normal Man by Neosynephrin* (1-α-hydroxy-β-methylamino-3-hydroxy-ethylbenzene hydrochloride).

ANCEL KEYS AND ANTONIO VIOLANTE.

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It is well known that heart rate and blood pressure do not rise in equivalent degree after administration of different sympathomimetic drugs. Ethylnor-suprarenin (3-4 dihydroxy-phenyl-1-amino-2-butanol-1) produces a rise in pulse rate and a fall in blood pressure (Cameron, et al.¹). Neosynephrin (3-hydroxyphenyl-1-methylamino-2-ethanol-1) increases the blood pressure with a relative fall in the pulse rate (Johnson²).

The production of relative bradycardia by sympathomimetic drugs has generally been ascribed to reflexes produced by the elevated blood pressure arising in the aortic arch and the carotid sinus. Such an effect can be demonstrated in man when small doses of epinephrine are used; the heart rate and blood pressure rise together but after some minutes the rate may fall while the blood pressure is still

^{*}This work has been supported by a fellowship grant to the University of Minnesota by Frederick Stearns and Company.

Minnesota by Frederick and M. M., Crismon, J. M., Whitsell, L. J., and Tainter, M. L., J. Pharm., 1937, 62, 318.

² Johnson, C. A., Surgery, Gyn., Obst., 1936, 68, 35.

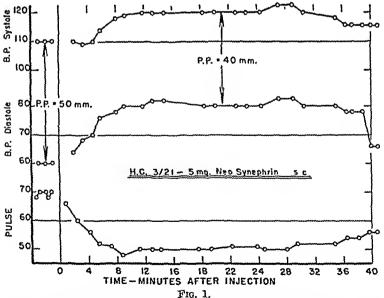
above normal. When continuous electrocardiographic records are made before, during and after injection of a small dose of epinephrine in man, we have found an immediate but very transient slowing of the heart (Fuchs³). Large doses of some of these drugs may produce ventricular bradycardia as a result of block, cryptosystole and general cardiac damage.

We have studied the cardiac and vasomotor responses in normal man to epinephrine, neosynephrin hydrochloride and synephrin tartrate (d- α -hydroxy- β -methylamino-4 hydroxy-ethylbenzene tartrate). All studies were made in basal rest on 14 trained subjects, who received subcutaneous injections at intervals of several days. Each subject was studied at various dosages of all 3 drugs.

Synephrine tartrate produced no effects on pulse, blood pressure or the electrocardiogram in doses up to 60 mg. Epinephrine produced the classical results as well as the very transient slowing and

occasionally the late reflex slowing mentioned above.

Neosynephrin consistently produced an immediate marked bradycardia which persisted from 30 to 90 minutes. Relative tachycardia never appeared except occasionally in very slight degree as the last effects of the drug were wearing off. The results were not changed



Typical course of blood pressure and pulse following subcutaneous injection of 5 mg of neosynephrin in a normal young man in the basal state.

³ Fuchs, R. T., J. Pharm., 1938, 63, 143.

when the subject was kept seated instead of prone. The threshold subeutaneous dose was from 1 to 2.5 mg in young adults from 110 to 180 lb in weight.

Fig. 1 shows the typical changes resulting from a rather small dose of neosynephrin. The pulse immediately starts to decline, reaching a constant low level at 7 to 10 minutes after injection. At the same time the diastolic blood pressure rises but the systolic pressure does not rise until the diastolic pressure and pulse changes are well established. With these rather small doses the systolic pressure rise is small and the pulse pressure is generally diminished. With a 10 mg injection (as is frequently used for the clinical dose) the initial time course is the same but the systolic pressure continues to rise so that the pulse pressure is eventually increased above normal. With the 10 mg dose the pulse rate may fall to 30 beats per minute and be maintained at 35 to 45 for as long as 80 minutes.

The electroeardiographic records are interesting. The rhythm is perfectly normal with no change in the PR interval though the RT interval (duration of systole) may be very slightly prolonged. There is no slurring of QRS in any of the leads at any time. Very rarely there may be inversion of P, especially in lead 3. Aside from the extreme bradycardia, the most notable change is a marked elevation of the T wave in all leads and a diminution of the P wave. In several cases the P wave practically disappeared and the E.C.G. would indicate A.V. nodal rhythm. Neither A.V. nor bundle branch block appeared. In a single ease when a 10 mg dose was given to a small athletic woman there was a short period when the E.C.G. record could be interpreted as showing retrograde conduction or complete A.V. dissociation at equal rhythm.

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Summary. Neosynephrin injected subcutaneously into normal, trained subjects in the basal state produces an immediate bradycardia and rise in diastolic pressure; systolic pressure rises later. The threshold is from 1 to 2.5 mg and pulse rates from 30 to 45, per-

⁴ Nathanson, M. H., Arch. Internal Med., 1936, 38, 683.

sisting for as long as 80 minutes, are produced by 5 to 10 mg. The E.C.G. remains normal with no change in A.V. or ventricular conduction time but there is a fall in the potential of the P wave and a rise in the T wave.

11339

A Method of Separating Small Quantities of the Coproporphyrin Isomers 1 and 3.

C. J. WATSON AND SAMUEL SCHWARTZ.

From the Division of Internal Medicine, University of Minnesota Hospital, Minneapolis.

Quantitative separation of the naturally occurring coproporphyrin isomers (1 and 3) has hitherto been impossible. Crystallization has usually permitted identification of the porphyrin predominating in any given mixture, such as obtained for instance from urine and feces.¹⁻⁴ This, however, has required that relatively large amounts of porphyrin be available. The present investigation was undertaken with the purpose of finding a means by which mixtures consisting of as little as 5-10 γ of total coproporphyrin could be resolved quantitatively.

We have found that the methyl esters of coproporphyrins 1 and 3 are quantitatively adsorbed on Brockmann's Al₂O₃* under the conditions noted in the following. The ester of coproporphyrin 3 may be eluted quantitatively with 35% acetone in water while that of copro-1 remains adsorbed, and is later removed by elution with pure acetone. The various steps in the procedure are as follows: (1) Esterification of the total, free porphyrin mixture in methyl alcohol saturated with HCl gas. (2) Dilution with equal volumes of distilled water, followed by neutralization of the HCl with a saturated aqueous solution of sodium acetate, which is added drop by drop with constant stirring until the solution no longer turns Congo paper blue. Ten percent NH₄OH is then added drop by drop until the mixture becomes pink to phenol red. (A few drops of an aqueous

¹ Watson, C. J., J. Clin. Invest., 1935, 14, 106.

² Watson, C. J., J. Clin. Invest., 1936, 15, 327.

³ Dobriner, K., J. Biol. Chem., 1936, 113, 1.

⁴ Watson, C. J., J. Clin. Invest., 1937, 16, 383.

^{*}Merck and Company, Inc.

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⁴ Nathanson, M. H., Arch. Internal Med., 1936, 38, 683.

metrically. In the present study a Zeiss stufenphotometer† has been used, and comparisons have been made with standard solutions of copro-3 ester in 35% acetone and copro-1 ester in pure acetone. The limit of error is within ±3%. The intensity of fluorescence is about twice that of the free porphyrin in 1% HCl. The chief objection to the measurement of red fluorescence with the stufenphotometer⁶ is that the eye fatigues rather rapidly and more than a few readings cannot be taken at any one time. Other methods of measurement are being investigated.

A summary of data obtained in a number of recovery experiments carried out with the above described method is given in Table I.

It is not possible as yet to report data on the application of the above method to natural material. We have ascertained that considerable purification of the free coproporphyrin is necessary, preliminary to esterification and subsequent separation of the isomers. Investigation is now in progress to determine as simple a method of purification as possible, which will still be generally applicable.

The Al₂O₃-acetone procedure is of much value in separating small amounts of copro-esters 1 and 3 for purposes of melting point determination and observation of crystal habitus. The data in Table II is evidence of the specificity of 35% acetone in eluting the copro-3. Extensive purification, consisting of repeated fractionation between ether and 1% HCl in the usual way¹ was carried out in each of these instances.

TABLE I.

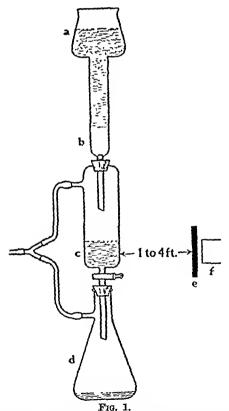
Recovery of Copro-1 and 3 Esters from Various Mixtures by Al₂O₃—Acetone Method.

No.	Amount Copro-1 used in \gamma	Amount Copro-1 recovered in	% recovery	Amount Copro-3 used in	Amount Copro-3 recovered in γ	% recovery	% Copro-1 in mixture
1	8.8	8.97	102	0.0	0.0		100
2	10.0	10.2	102	0.0	0.0		100
3	30.0	30.9	103	10.0	10.1	101	75
4	20.0	18.0	90	20.0	20.8	104	50
5	5.3	5.19	96	14.5	14.2	98	27
6	10.0	9.5	95	30.0	30.0	100	25
7	1.8	1.48	82	8.4	8.07	96	18
8	5.0	5.05	101	35.0	32,55	93	12,5
9	0.0	0.0		18.0	18.18	101	0.0

[†] The light source was a small, high pressure mercury arc lamp ("Mico" type) firmly attached to the front of the photometer. The light was filtered through a heat resisting red purple ultra filter, Corning No. 587.

⁵ Fikentscher, R., and Franke, K., Klin. Wchnschr., 1934, 922.

solution of the latter indicator having been added to the entire mixture.) (3) The faintly alkaline solution is at once run through the column of Brockmann's Al₂O₃, designated "a" in the accompanying diagram (Fig. 1). The column is next washed with 15-20 cc of distilled water. (4) The copro-3 ester is then removed by repeated washing with 35% acetone, as long as any red fluorescence is visible at b (Fig. 1). The total copro-3 fraction is collected in the lower suction flask and removed, after which elution with pure acetone is carried out in the same way. Relatively large amounts of 35% acetone are necessary for the copro-3 fraction. (5) The amount of porphyrin in each of the final solutions is then measured fluori-



Apparatus for adsorption and elution. a. Fluid from which porphyrins are to be removed. b. Column of Brockmann's Al_2O_3 (a cotton wad is inserted in the neck of the tube just below the Al_2O_3). c. Fluid to be inspected for red fluorescence in UV light. d. Receiving suction flask. e. Corning red purple ultra filter No. 587. f. Carbon are lamp.

11340

Polycythemia of Morbus Caeruleus (Cyanotic Type of Congenital Heart Disease.)

PHILLIP HALLOCK. (Introduced by C. J. Watson.)

From the Division of Internal Medicine, University of Minnesota Hospitals,

Minneapolis, Minn.

The polycythemia of morbus caeruleus is similar in many respects to that of the primary form (polycythemia vera), yet from an etiological point of view it differs a great deal. In both forms the red cell counts and hemoglobin concentrations are increased and the hematocrit readings are high. Blood volume studies have revealed that the total blood volume and circulating red cell mass are considerably elevated in polycythemia vera while the plasma volume remains essentially unaltered.1, 2, 3 While it is repeatedly stated that the total blood volume and red cell mass are elevated in the polycythemia of morbus caeruleus, these assertions are based not on actual blood volume determinations but on inferences drawn first, from the fact that the red cell counts and hemoglobin concentrations are high, and second, from the assumption that morbus caeruleus is similar to other forms of secondary polycythemia which arise from oxygen deficiency. Extremely meager information is available concerning the status of total blood volume, circulating red cell volume, and plasma volume in morbus caeruleus. The first case of morbus caeruleus in which total blood volume was measured and in which an attempt was made to estimate the plasma volume was that reported by Parkes-Weber and Dorner.4 In this case of morbus caeruleus the total blood volume, as determined by Haldane and Smith's carbon monoxide method, was found to be increased. The plasma volume was determined indirectly and considered to be normal from the estimation of the concentration of the dry residue in the blood serum. One year previous to this, however, Bie and Maar's had studied the concentration of plasma in 2 cases of morbus caeruleus by similarly estimating the concentration of the dry residue in the serum. They concluded that there was no significant change in plasma volume concentration

¹ Rowntree, L. G., and Brown, G. E., The Volume of Blood and Plasma in Health and Disease, W. B. Saunders Co., Philadelphia, 1929.

² Lampe, W., Deutsche Med. Wchnschr., 1925, 51, 2025.

³ Gibson, J. G., Harris, A. W., and Surgert, V. W., J. Clin. Invest., 1938, 18.

⁴ Parkes-Weber, F., and Dorner, G., Lancet, 1911, 180, 150.

⁵ Haldane, and Smith, L., J. Physiol., 1900, 25, 33.
6 Bie, W., and Maar, W., Deutsches Arch. fur Klin. Med., 1910, 44, 382.

forthold Mother	2
TABLE II.	Crystallization of Coproporphyrin Esters After Separation by Means of

Course. 2 agtor		M.P.* Crystal in °C habitus	191 Priging			152 Straight prisms Rheumatoud arthritis; Bom Lucially and rosoftes	157-61 Straight prisms Hodgkin's disease under xiny and rosettes	137-40 Straight prisms Rheumatic fever and rosettes
0.400	aribo	Amt in γ M (stufenphotometer)	1,000, 0,0	319 (89%)	302 (53%) 1	434 (81%)	534 (75%)	Relative amount only (57%)
orystatication of organization	cster	Crystal	Interior	Not crystallized	236.40 Fine eurving	Not crystallized	235 Rosettes of fine	curving necutes 242.4 Fine curving needles
Orysta	Copro-1 ester	Amt in y	No. (stufenphotometer) 111 C	40,3 (11%)	265.0 (47%)	3 88.0 (16%)	4 175.0 (25%)	5 Relative amount only (43%)

Blood Pindings in Marhus Caernleus (Cyanotle Typo of Congonital Meart Disease). TABLE I.

							B	Hood volume	,	Blood	Blond vol. per kg	kg			
			Ď	Ндв. %	R.B.C. I	Iomato.		Red cell	Total	Plasma	Red cell	Total	from	% devintion from normal volume	mo
	Λg_0	Sex	Kg,	100%	(Mill.) (Vol.%)	Vol.%)	Hters	Uters	liters			00	Plasma	~	Total
K.C.	18	×	0.00	120	9.0.6	83	!	8.03	10.4	29.5	1.1.1.0	173	-36	1539	1-30
A,N,		F	07.5	133	10.8	57		5,03	7.0	29.3	7.4.5	30,6	-25	•	1-40
I,W.B,	_	¥	20,8	128	6,8	7.7		4.95	0.0	27.6	82.8	110	-41	·	€; †
II.F.		M	45.5	137	0.0	73		4.10	5.7	33.0	01.5	126	81	Ī	£3
										30.0	98.3	128			
										TAR	UVE	ave			

K.C. = Tetralogy of Fallot. A.N. = ... L.W.B. = Patent ductus arterlosus with patent interventricular septal defect. II.F. = Infundibular stenosis with Interventricular septal defect.

and that the increased cell count was not, therefore, due to diminution in plasma volume. Blumenfeldt and Wolheim, using trypared dye estimated the blood volume in a 20-year-old girl with cyanotitype of heart disease. The plasma volume was markedly diminished namely, 690 cc (13.4 cc per kg). The total blood volume was 3,02 cc or 59.1 cc per kg. Meyer, in a study of the hemodynamics of the circulation in a 22-year-old male having Tetralogy of Fallot, estimated the plasma volume by means of the Congo red method and found it to be exceedingly low (755 cc or about 16 cc per kg). The total blood volume was 4,100 cc, or about 86 cc per kg, which is within normal limits.

In the present investigation, 4 cases of morbus caeruleus have been studied with respect to total blood volume, circulating red cell volume and plasma volume. The subjects were adults, 3 males and one female, all of whom had veno-arterial shunts as evidenced by the marked cyanosis. Their cardiac status was carefully studied by physical examination and by fluoroscopic and electrocardiographic examinations of the heart. The oxygen content and capacity were determined by arterial puncture and in every instance oxygen unsaturation was increased. The "Evans Blue" dye was employed in determining plasma volume following the method described by Gibson. The dye concentration in the serum was determined with a Marten's spectrophotometer.

Results. It will be noted (Table I) that the hematocrit values are extremely high when it is remembered that the normal range is 45 to 50% for males and 40 to 45% for females. The highest value, 83%, recorded in this series was in a male of 18 years of age who was believed to have Tetralogy of Fallot. The lowest value, 72%, was obtained in a female 37 years of age who suffered from the same anomaly. The most striking feature (Table I) was the low absolute plasma volumes which were consistently found in all 4 cases. The lowest plasma volume (27.6 cc per kg) was obtained in the third case, a male 33 years of age. The average plasma volume in the 4 cases was 30 cc per kg.

The circulating red cell mass and total blood volume were found to be elevated in each instance.

In Table II we have compared the results obtained in morbus caeruleus with those of normal individuals and those with primary polycythemia (polycythemia vera). It will be noted that in morbus

⁷ Blumenfeldt, E., and Wolheim, E., Klin. Wschr., 1927, 6, 396.

⁸ Meyer, P., Z. fur Klin. Med., 1932, 120, 341.

⁹ Gibson, J. G., J. Clin. Invest., 1937, 16, 301.

Blood Findings in Morbus Caeruleus (Cyanotic Type of Congenital Heart Disease). TABLE I.

	omi	Total	+90	+40	+33	+30			
	% deviation from normal volumo	Red cell	+230	+100	+ 92	+110			
	from from	Plasma]	-36	-25	-41	-28			
g	Total	99	173	104	110	125		128	AVE
vol. per l	Plasma Red cell Tota	66	144.0	74.5	82.8	91.5	-	98.5	ave
Blood	Plasma	00	29.5	29.3	27.6	33.0	-	30.0	JAU
•	Total	liters	10.4	7.0	9.9	5.7			
Blood volumo	Red cell	vol. liters	8.63	5.02	4.95	4.16			
P		ros. liters	ł						
	Hemato.	per mms eric (Mill.) (Vol.%)	83	72	74	73			
	R.B.C.	per mm. (Mill.)	9.04	10.8	6.8	6.5			
	Hgb. %	100%	126	133	128	137			
	Ē	re re	60.0	67.5	59.8	45.5			
		Sex	¥	F	¥	M			
		Age	18	37	33	43			
			K.C.	A.N.	I.W.B.	H.F.			

K.C. = Tetralogy of Fallot.
A.N. = I.W.B. = Patent ductus arteriosus with patent interventricular septal defect.
H.F. = Infundibular stenosis with interventricular septal defect.

TABLE II.

Comparison of Blood Volume in Morbus Caeruleus (Cyanotic Type of Congenital Heart Disease) to That in Polyeythemia Vera and in Normal Individuals.

Pi	asma volume ee per kg	Red cell volume cc per kg	Total blood volume cc per kg
	Females		
Normal (avg of 10 normals)	39.0	32.0	70.8
Morbus eneruleus	29.3	74.5	104.0
Polycythemia vera (avg of 6 eases	s) 76.7 Males	177.0	253.7
Normal (avg of 10 normals)	46.4	43.1	89.5
Morbus eneruleus (avg of 3 eases	30.3	109.0	136.0
Polycythemia vera (avg of 6 case		145.0	196.2

caeruleus the plasma volume is 29.3 cc per kg as compared to the mean normal of 39 cc per kg for females and 30.3 cc per kg as compared to a mean normal of 46.4 cc per kg for males. This represents a subnormal diminution of plasma volume from the mean normal by 26 and 35% respectively. When the plasma volume of morbus caeruleus is compared with that of polycythemia, it will be noted that the latter shows a definite increase even over the normal.

When the circulating red cell volume of morbus caeruleus is compared with the normal and with that in polycythemia vera (Table II) it is noted that for both males and females the red cell volume is decidedly increased in morbus caeruleus and strikingly increased in polycythemia vera.

The total blood volume is but moderately increased in morbus caeruleus while in polycythemia vera it is greatly increased. In one instance the increase was 300% (Fig. 1). This patient had a total blood volume of 18.28 liters of blood, approximately 3 times greater

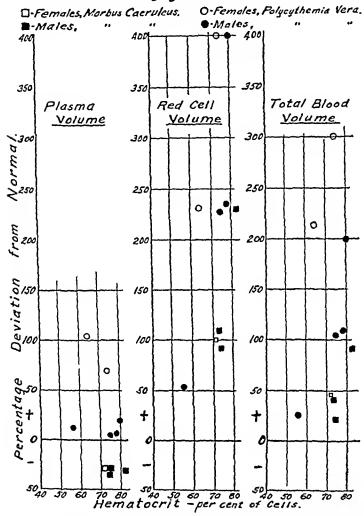
than normal.

In Fig. 1 it is noted that the plasma volumes of morbus caeruleus are all subnormal, a percentage deviation of -36%, -25%, -41%,

and -28% respectively below the mean normal.

Summary. The results of the present study indicate that the plasma volume is subnormal in adult cases of morbus caeruleus while the total circulating blood volume is moderately increased, this increase being due to a considerable increase in circulating red cell volume. From the viewpoint of the disturbed dynamics of the circulation in morbus caeruleus, these blood volume findings would appear to indicate a compensatory effort on the part of the circulation to maintain a normal blood volume at the expense of a reduced plasma volume. At the same time, this permits the increase in circulating red cell volume which is necessary to compensate in part at least for the anoxia.

Fig.I. Comparison of Blood Volume in Morbus Caeruleus to that in Polycythemia Vera.



The blood volume findings in morbus caeruleus resemble those of polycythemia vera in that in both conditions the circulating red cell volume and total volume are increased both in relative and absolute values. The only difference is associated with the plasma volume change; in morbus caeruleus it is subnormal while in polycythemia vera the plasma volume tends to be slightly above normal.*

^{*} Studies in two of the above cases were made possible through the courtesy of Dr. O. N. Nelson of the Minneapolis General Hospital.

11341 P

Effect of Epinephrine on Potassium Balance in the Perfused Hind Limbs of the Frog.

J. CLIFFORD STICKNEY. (Introduced by Ancel Keys.)

From the Laboratory of Physiological Hygicne, University of Minnesota Medical School, Minneapolis, Minn.

Intravenous injection of epinephrine produces an immediate transitory rise in the level of plasma potassium.^{1, 2, 3} The duration and magnitude of this effect differs in different animal species and a subsequent fall in [K]s to below normal is equally marked and less transitory, especially in man.⁴ The initial rise in [K]s seems to originate in the liver² but it appeared possible that the skeletal muscle might be involved in the slower and more sustained decline.

Perfusion preparations of the isolated hind limbs of the double-pithed frog were made. The perfusion fluid was a 3% gum acacia solution with the salt content and pH adjusted to correspond with normal frog Ringer's solution except for K which was somewhat high (5.18 to 6.03 m.eq./1). A perfusion pump supplied pulsating pressure to the inflow cannula entering the terminal aorta. The outflow was collected from cannulae in the renal portal veins, all other egress being prevented by ligatures. [K] was determined in arterial and venous samples collected at intervals during a period of $2\frac{1}{2}$ to $3\frac{1}{2}$ hours. Rates of flow were measured throughout.

In preliminary experiments single injection of epinephrine into the arterial inflow gave somewhat inconstant but essentially negative results with regard to the [K] in the venous outflow. In all cases the [K] in the arterial inflow was the most important factor in determining the direction and rate of K exchange between the tissue and the perfusion fluid. When epinephrine was continuously infused into the arterial inflow (0.005 mg/cc) there was consistently a marked effect on the arteriovenous K difference, but almost no effect on the rate of K exchange between the tissue and the perfusing fluid. In other words, in these experiments, the movement of K from vascular bed to tissue proceeded at a rate independent of the total rate

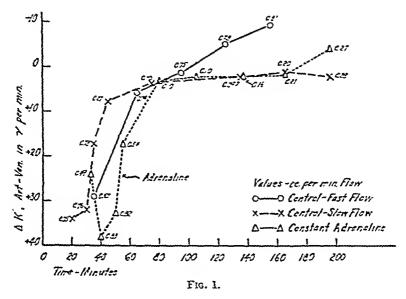
¹ D'Silva, J. L., J. Physiol., 1934, 82, 393.

² Marenzi, A. D., and Gerschman, R., Rev. Soc. argent. de biol., 1936, 12, 424.

³ Brewer, G., Larson, P. S., and Schroeder, A. R., Am. J. Physiol., 1939, 126, 708.

⁴ Keys, Ancel, Am. J. Physiol., 1938, 121, 325.

⁵ Hartzler, E. R., J. Biol. Chem., 1937, 122, 19.



of flow. This was true whether the rate of flow was altered by epinephrine administration or by simple change in the perfusing pressure which was studied in separate experiments. These points are shown in Fig. 1 in which typical results are plotted.

These results show that when a small K gradient from blood vessel to tissue is applied in resting muscle, the rate of renewal of the blood phase is not, within physiological limits, a limiting factor for the K exchange, nor could a direct effect of epinephrine be seen. Further experiments with no net movement of K in control periods likewise failed to demonstrate a direct effect of epinephrine on K exchange or balance in resting muscle.

11342

Elimination of Radioactive Elements in Patients Who Have Received Thorotrast Intravenously.

WILHELM STENSTROM AND IRWIN VIGNESS.

From the Department of Radiology, University of Minnesota.

The radioactive method of determining the elimination of any elements in the thorium series is more sensitive than the chemical method and has been used exclusively in this study which deals with the elimination from patients who previously have received intravenous injection of thorotrast. Most of the ionization produced is caused by α -particles and preliminary studies indicated that an electroscope was the most suitable instrument for the detection of the ionization. The limit of the sensitivity of the method used was such as to detect 0.005 cc of thorotrast mixed with 4 g of ash. The γ -ray Geiger-Müller counter did not give a reliable response to less than 1 cc of thorotrast at 1 cm distance.

Apparatus and Method. A Wulf bifilar type of electrometer, which has a low capacity, was used in conjunction with a cylindrical ionization chamber 18 cm in diameter and 26 cm long. The chamber was attached directly to the electrometer.

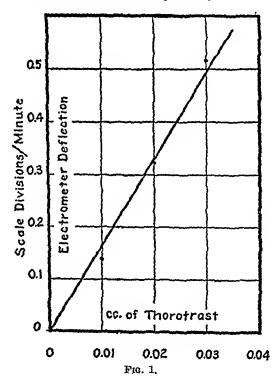
In order to obtain a measure of the thoron in the breath, a patient exhaled through a rubber tube into a closed cylindrical metal container having a diameter of 30 cm and a height of 30 cm. Several small holes in the cover provided for the escape of the exhaust air. A copper wire with an active length of 21 cm extended along the axis of the container. It was insulated from the grounded container and kept at a negative potential of about 2000 volts and served to collect the radioactive deposit caused by the disintegration of the thoron. After the collection had been made for a time, the wire was transferred and used as the central collecting electrode of the ionization chamber on top of the electrometer. The lengths of time that the patients breathed into the container were between 1 and 2 hours, but more deposit would build up if this could be continued for longer time (up to 20 or 30 consecutive hours). The half life time of thoron is only 55 seconds and that of thorium A 0.1 second. next element, thorium B, has, however, a half life time of 10.6 hours and will therefore make the wire active for a relatively long time after it has been deposited.

The stool was collected in a waxed cardboard container. This box with its content was placed in an iron (sand bath) dish and heated

for about 5 hours until a light colored ash remained, usually weighing from 2 to 4 g. The ash was then spread evenly over a circular, light cardboard paper about 17 cm in diameter and having a central hole 4 cm in diameter.

The ash obtained from the urine was a heavy black fusable material which probably consisted of carbon contained in various salts. This ash was so bulky and heavy that the α -rays were largely absorbed and the unsatisfactory results obtained indicated that it will be necessary to extract the radioactive substances from the urine when the studies are continued.

Results. In order to obtain an idea of the sensitivity of the method, small amounts of thorotrast were mixed with 4 g of ash which then was heated and thoroughly stirred. Measurements of the radioactivity of ash containing different amounts of thorotrast are reproduced graphically in Fig. 1, where the discharge of the electroscope in scale divisions per minute is plotted against the known amount of thorotrast present in the ash. The 3 dots correspond to 0.01, 0.02, and 0.03 cc of thorotrast respectively.



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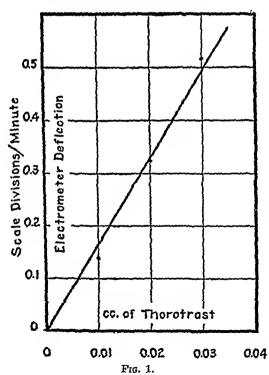
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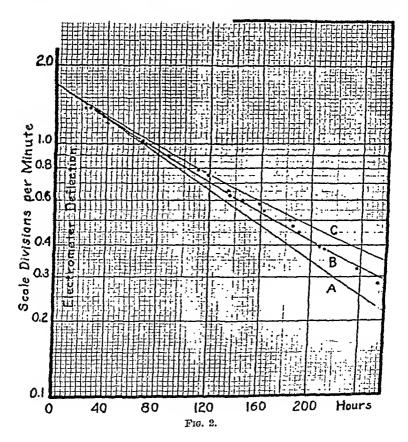
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Examinations were made of the ashed feces from 2 patients who had intravenous injection of thorotrast 6 and 7 years previously. Each one had been given 75 cc of thorotrast which contained 24 to 26% thorium dioxide by volume. Easily measurable radioactivity was found in all the samples. When the samples were reëxamined several weeks later, it was, however, found that the radioactivity had almost completely disappeared. It was, therefore, assumed that most of the radioactivity was due to elements with short lifetime belonging to the thorium series. A stool obtained from the second patient at a known time was then ashed as soon as possible and its activity studied as a function of time. The results are plotted in Fig. 2. The points represent the number of scale divisions the electroscope was discharged per minute at different hours from the time the stool was collected. Curves A, B, and C were calculated from the known



disintegration constants. Curve A shows the relation between radioactivity and time if only thorium X (half life time 87.4 hours) in equilibrium with its disintegration products was present, curve B shows the decay of a mixture of 95% thorium X and 5% of some preceding long lived product of the thorium series (thorium, mesothorium or radiothorium), and curve C illustrates a mixture of 91% thorium X and 9% long lived element. The radioactive elements present in the feces in this case seem to consist of approximately 95% thorium X and 5% of long lived elements, each in equilibrium with its disintegration products. A slight change in the normal background drift of the electrometer could. however, account for the difference between the curve representing thorium X and the experimental points. Measurements 4 months later indicate that 2 to 3% of the activity was due to long lived elements in equilibrium with their disintegration products.

Several samples of ashed feces from other individuals, who had never had any thorotrast injected, were also examined but in no case was any radioactivity discovered.

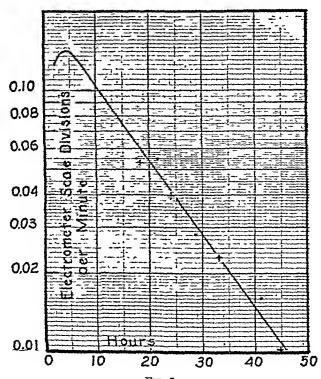


Fig. 3.

One sample of ashed urine from the second patient showed slight radioactivity which decayed with time. The patients were discharged before urine could be obtained for an attempt to extract the radioactive substances.

No radioactivity was discovered in one sample of sputum which had been collected over a period of 24 hours and then ashed. More sputum examinations ought to be done.

The results from one of the breathing experiments are shown in Fig. 3. The points fall very close to the theoretical curve for disintegration of thorium B and it is, therefore, evident that a fair amount of thoron is exhaled by the patient. This must mean that the thoron can pass rapidly from the source (probably liver and spleen) to the breath as the half life time for the thoron is only 55 seconds.

Summary. No excretion of thorium has thus far been discovered. Certain radioactive elements in the thorium series have, however, been found in the feces, urine and breath. Thorium X has been identified as the predominant element excreted in the feces, and thoron is definitely exhaled, as thorium B has been identified in the radioactive deposit from the breath. Such excretion of thorium X and thoron leads to reduced radioactivity in the body even if the thorium itself remains. As most of the γ -rays are emitted by disintegration products of thoron it is evident that the amount of thorium remaining in the tissues of a patient can not be determined with satisfactory accuracy by measurements of the γ -rays emitted from the patient (liver and spleen). During the disintegration process of some of the radioactive atoms such a displacement must take place that the newly formed atoms can escape.

11343 P

Influence of Pregnancy and Lactation on Susceptibility to Arrest of Brain Circulation.*;

HERMAN KABAT. (Introduced by M. B. Visscher.)

From the University of Minnesota, Minneapolis.

By means of a new technic, it has been possible to obtain constant results from dog to dog with the same period of complete arrest of the brain circulation. The sex, age, or breed of the experimental adult animal had no influence on the sensitivity of the brain to arrest of its blood flow. On the other hand, puppies were much more resistant than adult animals to this procedure. The present report deals with the influence of pregnancy and lactation on the resistance of the brain to arrest of its circulation.

In every instance, the pregnant or lactating animal was more severely affected by brain stasis than the normal adult animal. A similar increase in susceptibility was shown by 2 young females at about the age of sexual maturity, one of whom was in the first oestrus. The results are compiled in Table I. Only the dogs indicated by asterisks (after survival) died from the arrest of the brain circulation. The others were either sacrificed or are still alive. The striking increase in severity of the brain damage as compared to normal adult animals is evident.

A study of the time of persistence of respiration and the corneal reflex following acute arrest of blood flow in the brain shows no difference between pregnant or lactating and normal adults. Furthermore, the recovery times of respiration and the corneal reflex following restoration of cephalic blood flow were very similar in the 2 groups of dogs. In other words, the survival time and the recovery time are within normal limits while the revival time⁸ is greatly decreased by pregnancy and lactation. This suggests the possibility that the greater susceptibility during pregnancy and lactation may be due, not to a difference in the rate of metabolism, but rather to a difference in the ability of the animal to overcome reversible neuronal damage.

^{*} Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[†] Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration, Official Project No. 665-71-3-69, subproject No. 309.

¹ Kabat, H., and Dennis, C., Proc. Soc. Exp. Biol. and Med., 1938, 38, 864.

Kabat, H., and Dennis, C., Proc. Soc. Exp. Biol. And Med., 1939, 42, 534.
 Gerard, R. W., Arch. Neurol. and Psychiat., 1938, 40, 985.

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33 33 34	Apparently permanently blind, deaf, anosmic. Decreased	responsiveness to pain. No attempt to stand or walk. Very little spontaneous activity. No emotion or vocalization. Retains taste, ability to feed himself, and various re-	flexes No recovery from coma. Even unable to lap milk, which normal dog can do following 10-minute period of brain stans	No recovery from coma, Drinks milk when snout is placed in it. Complex responses in urination and defection.
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ulation.		End result and comment	Apparently complete recovery	11 11 11 11 11 11 11 11 11 11 11 11 11	No recovery from coma	Apparently complete recovery	Apparently complete recovery	No recovery from coma	Apparently complete recovery	Permanent clumsy gait, stow	movements, little spontaneous activity, little emotional ox-	Apparently complete recovery	33 33	Litterunato of BK20 No recovery from coma
Brain Cire		Survival	om 4	, 2 ; %''	3 days*	61	3 mo	6 days	3 mo	Still alive	(21112)	Still alivo	3 110 110	9 days
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	Dog	BK14	BK22 18	BK16	17	BK3	BK17 BK13	La 1	La. 2		BK15	BK20 BK21	BK18	

The method of sampling, storing, extracting, and quantitative analysis has previously been described by Miller and Johnson.⁵

The analytical data are presented in Table I of total chlorophyll concentration expressed as percent on a green weight basis and as milligrams per 100 sq cm of leaf surface in 15 crosses between high chlorophyll female X low male and reciprocally in low chlorophyll female high male. The values reported are the average of 2 field replications grown in 1939. The inbred parents used in these crosses differed significantly in percent total chlorophyll; the highest chlorophyll inbred parent of the low chlorophyll lines being significantly lower than the lowest parent in the high chlorophyll lines as measured by the analysis of variance in 2 field replications in 1938 and 1939.

The transmission of variegation (unequal distribution of chlorophyll in the leaves) in *Mirabilis* occurs only through the egg cell cytoplasm. This has raised the question, whether any plastids are transmitted in the cytoplasm contributed by the male parent; and if there are, of what importance is such a transmission, in comparison with that contributed by the female.

During fertilization, according to Anderson, it is possible for plastids in the cytoplasm to be carried along with the male nucleus when the pollen tube ruptures. In *Antirrhinum*, during the time that the male nucleus migrates through the cytoplasm of the embryo

TABLE I.

% Total Chlorophyll and mg of Total Chlorophyll per 100 cm² of Leaf Tissue in Reciprocal Crosses Between High and Low Chlorophyll Inbred Lines of Corn.

			l chloroj 1 crosses		Mg chlore F	phyll per 1 crosses	r 100 cm²
Cross	Inbred parents	HzL	LxH	Dif.	HxL	LzH	Dif.
6 x 23	.319 x .352	.367	.286	.81	7.12	5.78	1.34
x 26 x 44	x .360 x .361	.305 .360	.289 .357	.16 $.03$	$6.64 \\ 6.82$	$6.20 \\ 6.63$.44 .19
x 48 11 x 23	x .364 .232 x .352	.321 .299	.328 .296	07 .03	$6.28 \\ 6.16$	$6.70 \\ 6.01$	42 .15
x 26 x 44	x .360 x .361	.274 .330	.279 .342	05 12	6.00 6.52	6.30 7.06	30 54
x 48	x .364	.288	.319	31	5.93	6.51	58
34 x 23 x 26	.273 x .352 x .360	.331 $.322$.326 .282	.05 .40	7.04 7.50	$6.66 \\ 6.63$.38 .87
x 48 45 x 23	x .364 .352 x .352	.299 $.342$.299 .356	.00 14	$6.53 \\ 6.51$	$6.45 \\ 6.56$.08 05
x 26 x 44	x .360 x .361	.299 .361	.313 .356	14 .05	6.15 6.50	$6.44 \\ 7.03$	29 53
x 48	x .364	.386	.375	.11	6.93	6.93	.00
	Mean	326 $Z = .5$.320 21 Odd	05400 $1s = 3.4$		6.52 093 Od	049333 $ds \approx 1.7:1$

⁵ Miller, Elmer S., and Johnson, I. J., Am. Soc. Agron., 1938, 30, 941.

11344

Inheritance of Chlorophyll in F₁ Crosses Made Reciprocally Between Selfed Lines of Corn.*

Elmer S. Miller and I. J. Johnson.†

From the Department of Botany, Agronomy and Plant Genetics, University of Minnesota, Minneapolis.

The inheritance of chlorophyll variations in plants has been investigated in detail; over one hundred chlorophyll abnormalities have been reported in Maize. Correns' suggested that chlorophyll deficiencies were transmitted as inclusions and plastids through the cytoplasm of the female parent during fertilization, but that none was transmitted from the male parent in the cytoplasm, along with the male nucleus to the egg. The classical example is the transmission of variegation in Mirabilis only through the cytoplasm of the egg, but not along with the male nucleus. These findings have been confirmed by Baur² and are supported by East.³

Recently Anderson has reported the results of a series of experiments and he concludes:

- a. Plastids are identical and numerous in both female and male gametophytes.
- b. In the pollen tube, numerous plastids were always present near the male nuclei, and as the tube is ruptured, the plastids are expelled into the eytoplasm of the embryo sac.
- c. The earlier investigators fixed and preserved samples in such a manner that all the inclusions were dissolved.

In this study, the writers have on a physiological basis set up the following experiment to further determine whether maternal inheritance, as transmitted by cytoplasm of respective parent to F_1 , is of any importance in inheritance studies of the chlorophyll pigments.

^{*}Contribution from the Department of Botany and Division of Agronomy and Plant Genetics, University of Minnesota. Paper No. 1776 of the Journal Series. Aided by a grant from the Graduate School of the University of Minnesota. Assistance in the preparation of these materials was furnished by the personnel of Works Projects Administration Official Project No. 65-1-71-140, Sub-project No. 331.

[†] Now Professor and Research Professor of Farm Crops, Iowa State College, Ames, Iowa. Formerly in Agronomy and Plant Genetics, University Farm.

¹ Correns, G., Z. Ind. Abst. Vererb., 1909, 1, 291.

² Baur, E., Z. Ind. Abst. Vererb., 1909, 1, 330.

³ East, E. M., Am. Nat., 1934, 63, 289; 402.

⁴ Anderson, Lewis F., Am. J. Bot., 1936, 23, 490.

The method of sampling, storing, extracting, and quantitative analysis has previously been described by Miller and Johnson.⁵

The analytical data are presented in Table I of total chlorophyll concentration expressed as percent on a green weight basis and as milligrams per 100 sq cm of leaf surface in 15 crosses between high chlorophyll female X low male and reciprocally in low chlorophyll female high male. The values reported are the average of 2 field replications grown in 1939. The inbred parents used in these crosses differed significantly in percent total chlorophyll; the highest chlorophyll inbred parent of the low chlorophyll lines being significantly lower than the lowest parent in the high chlorophyll lines as measured by the analysis of variance in 2 field replications in 1938 and 1939.

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Cross	Inbred parents	HxL	LхH	Dif.	HxL	LxH	Dif.
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x 44 x 48	x .361 x .364 Mean	.361 .386 .326 Z = .3	.356 .375	0.05 0.05400 0.05400 0.05400	6.50 6.93 6.57	6.44 7.03 6.93 6.52 093 Odd	29 53 .00 .049333 ds = 1.7:1

⁵ Miller, Elmer S., and Johnson, I. J., Am. Soc. Agron., 1938, 30, 941.

sac, and while the male nucleus is in the process of fusing with the polar nuclei to form the endosperm nucleus, there are mitochondria in the vicinity of the endosperm nucleus. These mitochondria originally came from both the male and female parents.

Thus, on a cytological basis, it appears that there are species differences regarding the mechanism and importance of cytoplasmic inheritance. In *Mirabilis*, it is important with respect to leaf variegation, but in other cases, i. e., corn, with odds as low as 3.4 to 1 and 1.7 to 1 for percent total chlorophyll and milligrams chlorophyll per 100 sq cm leaf surface respectively, as measured by "Students" pairing method and tables of Z, it is apparent that cytoplasmic inheritance is not an important factor in inheritance studies of chlorophyll in corn.

Summary. These studies show that maternal inheritance of chlorophylls as measured by a series of crosses made reciprocally between high and low chlorophyll inbred lines of corn is not significant, and that male and female parents each contribute equally to the genotype of the F_1 cross in respect to chlorophyll concentration.

11345

In vitro Experiments on Exchange of Phosphate by Enamel and Dentin.

W. D. Armstrong. (Introduced by F. H. Scott.)

From the Laboratories of Dental Research and Physiological Chemistry, University of Minnesota, Minneapolis, Minn.

Krogh, Holst, and Hevesy,¹ and Manly and Bale² demonstrated the presence of radioactive phosphorus in the whole teeth of animals receiving a parenteral administration of compounds of this isotope. Hevesy and Armstrong³ in an investigation in which the enamel and dentin of cats' teeth were separately studied found, using radioactive phosphorus as an indicator, the rate of exchange of phosphate by the enamel of erupted mature teeth to be about one-tenth that of the dentin. The rate of exchange was such as to make highly improbable an ability of enamel of mature teeth to undergo significant changes of composition as a result of nutritional alterations.

¹ Krogh, A., Holst, J. J., and Hevesy, G., Det. Kgl. Danske Videns, Selskab. Biol. Med., 1937, 13, 13.

² Manly, L., and Bale, W. F., J. Biol. Chem., 1939, 129, 125.

³ Hevest, G., and Armstrong, W. D., Proc. Am. Soc. Biol. Chem., 1940, XLIV.

The experiments herewith reported were carried out by agitating 0.12-0.15 mg of the finely pulverized specimens in 10 cc volumetric flasks completely filled with the solution of labeled phosphate. active material was supplied by the Radiation Laboratory of the Department of Physics of this University. The specimens after the stated time of contact with the active solution were recovered and washed ten times with water by centrifugation.

These results obtained in vitro, especially in the case of dentin, show a surprisingly rapid rate of exchange of phosphate. However, the relative rates of exchange by enamel and dentin are very similar to those observed in vivo by Hevesy and Armstrong.3

Since dentin contains 22,2% protein and enamel less than 1% of protein, the larger amount of labeled phosphate acquired by dentin from solutions at pH 7.5 might have been due, if the protein of dentin has an isoelectric point somewhat above pH 7.5, to combination of phosphate anion with dentin protein. The acid combining power of dentin protein in relation to hydrogen ion concentration is not known but it appears very unlikely that this protein could combine with anions in solutions of pH 13. The experiments whose results are tabulated in the second and third columns of Table I indicate almost identical rates of acquisition of labeled phosphate by dentin from a solution of pH 7.5 and 13. It is, therefore, unlikely that the higher rate of uptake of radioactive phosphate by dentin at pH 7.5 was due to chemical combination of phosphate with dentin protein. The 2 experiments with dentin at pH 7.5 and 13 also indicate no effect of the state of ionization of phosphate on its rate of exchange with dentin

> TABLE I. Exchange of Phosphorus by Enamel and Dentin.

Material	Enamel	Dentin	Dentin	Dentin Protein
pH solution	7.42	7.51	13	7.4
Time in hr at 38°	20	20	22.5	20
Activity (counts/min)	19.6 ±0.33	111.0 ± 1.6	218 ±1.9	8.85±0.28
Background (counts/min)	8.62 ± 0.21	8.62 ± 0.21	8.80±0.1	7 8.80±0.17
Mg P in solution	18.5	18.5	18.5	14.0
Mg P labeled by 1 count*	0.0122	0.0122	0.0114	
Mg P exchanged/g/24 hr	1.42	11.7	11.8	
% P exchanged/24 hr	0.81	9.19	9.26	
Specific activity!	0.0445	0.502	0.505	
Relative specific activity	1	11.2	11.3	

^{*} Calculated from total activity of solution and its content of phosphorus.
† Specific activity: The % of total activity in a tissue per mg of phosphorus.
4 Armstrong, W. D., Brekhus, P. J., and Cavett, J. W., J. Dent. Research, 1936, 15, 312,

As further evidence that dentin protein does not combine with phosphate at pH 7.5, denatured dentin protein was agitated for 20 hours with a solution of labeled phosphate with the result that no active phosphate was present in the protein after thorough washing with water.

The greater rate of exchange of phosphate by dentin must, therefore, be attributed to the smaller size of the crystallites of the mineral phase of dentin, but more especially to the fact that dentin is permeated by the dentinal tubules which probably have the effect of permitting a more complete contact of the crystals of the mineral phase with the solution containing labeled phosphate than exists in the case of enamel. An alternative hypothesis might be that the higher activity of dentin is due merely to some active solution trapped in the dentinal tubules. Nevertheless, phosphate in solution which is not removed from the tubules by thorough washing would be expected, with time, to reach an exchange equilibrium with the phosphate of the mineral phase.

11346

Cinnamic Acid Metabolism in Man.

I. SNAPPER, T. F. Yü AND Y. T. CHIANG. (Introduced by S. H. Liu.)

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The fate of cinnamic acid (C₀H₂CH:CHCOOH) after its administration to the animal organism has been examined repeatedly. The following facts are known. Cinnamic acid given by mouth to humans is oxidized to benzoic acid which is excreted in the urine linked with glycine in the form of hippuric acid.^{1, 2} In cats and dogs after the administration of phenylpropionic acid not only hippuric acid but also small amounts of cinnamoylglycine are excreted.³ After administration of cinnamic acid hippuric acid with small traces of cinnamoylglycine are found.⁴ After administration of cinnamic

¹ Erdmann and Marchand, Liebig's Annalen der Chemie und Pharmacie, 1842, 44, 344.

² Knoop, F., Beiträge zur Chemischen Physiologie, 1905, 6, 150, and 1908, 11,

³ Dakin, H. D., J. Biol. Chem., 1906, 5, 173, 303.

⁴ Dakin, H. D., J. Biol. Chem., 1907, 6, 203.

acid by mouth to dogs 65-70% of the excreted benzoic acid is present in the form of benzoylglycuronic acid and 30% in the form of hippuric acid. The isolated kidney of calves and sheep is able to oxidize cinnamic acid to benzoic acid during perfusion. However, the isolated kidney of the dog is not able to oxidize cinnamic acid but only conjugates the cinnamic acid with glycine to form cinnamoylglycine. Excretion of cinnamoylglycuronic acid has not been observed previously. We have reexamined the question whether after cinnamic acid administration to humans free or conjugated cinnamic acid could be found in the urine.

Experimental. Six g of cinnamic acid were given after dissolving in 200 cc of water and neutralizing. The urine excreted within the first 4 hours of the experiment was examined in order to compare the results obtained with Quick's hippuric acid test.

- 1. Glycuronic acid was excreted in considerable amounts. The urine always gave a positive naphthoresorcin reaction. The amount of glycuronic acid varied between 300 and 800 mg determined with the Shaffer-Hartmann technic.
- 2. Hippuric acid. To the 4 hours' urine concentrated HCl was added until the reaction became acid to Congo red. A considerable precipitate (4-5 g) was formed consisting of hippuric acid. After standing in the icebox for some hours the precipitate was filtered off. The filtrate still contained about 0.330 g of hippuric acid per 100 cc.
- 3. Presence of free cinnamic acid. Only small amounts of free cinnamic acid were excreted in this experiment. After chloroform extraction of the acidified urine. 10-15 mg of free cinnamic acid were demonstrated in the extraction fluid.
- 4. Presence of a cinnamic acid compound. Concentrated NaOH was added to the urine filtrate until the reaction was frankly alkaline. The alkaline fluid was boiled for one hour over a free flame and was then evaporated on a waterbath to a volume of 10-30 cc. After cooling, strong HCl was added. As soon as the reaction became acid, a thick white precipitate formed. After a few hours in the icebox, this precipitate was filtered off and dried in an incubator overnight. Next morning the precipitate was extracted on the waterbath for 2 hours with 150 cc of chloroform under a reflux con-

⁵ Quick, A. J., J. Biol. Chem., 1928, 77, 581.

⁶ Snapper, I., and Grünbaum, A., Bioch. Z., 1924, 150, 12.

⁷ Snapper, I., and Grünbaum, A., Acta brevia Neerlandica, 1934, 4, 38, and Pharmaceutisch Weekblad, 1934, Jubileumboek Prof. P. van der Wielen.

⁸ Tollens, B., Allen's comm. Organic Analysis, 5th edition, 1, 496, and Ber. d. Deutsch. chem. Ges., 1908, 41, 1788.

⁹ Quick, A. J., J. Biol. Chem., 1926, 69, 555.

denser. The chloroform was then filtered into a separatory funnel and extracted twice with 5 cc 8% NaOH.

To this alkaline solution, concentrated HCl was added until precipitation occurred. The precipitate was filtered off and dried. This precipitate contained a considerable amount of cinnamic acid. A. If, to a few mg of these crystals, one drop of Na₂CO₃ and one drop of KMnO₄ solution were added, the KMnO₄ quickly turned brown by reduction and a strong smell of benzaldehyde developed. B. By titration with bromine, considerable amounts of cinnamic acid were determined. For this titration a combination of the methods published by A. W. K. de Jong¹⁰ and by Greenberg and Mackay¹¹ was used. C. Pure cinnamic acid crystals with a melting point of 133°C were recovered by recrystallization from boiling petroleum ether.

5. Presence of cinnamic acid as monocinnamoylglycuronic acid. As the cinnamic acid compound present in the urine was very soluble in water, the possibility of the presence of cinnamic acid conjugated with glycuronic acid had to be considered. The following experiment makes the presence of a cinnamoylglycuronic acid compound seem probable.

The filtrate obtained after acidifying the urine with HCl was extracted with ether in a continuous Lind extractor for 15-20 hours. At that time the other in the extraction flask showed a watery layer. This watery layer was separated from the other and transferred to a 25 cc volumetric flask. After addition of 2 cc concentrated HCl, the volume was made up to 25 cc.

This solution was strongly reducing. Two samples of 4 cc each were used for the quantitative determination of glycuronic acid (Shaffer-Hartmann method). The remaining 17 cc were boiled for 30 minutes under a reflux condenser and then transferred while still hot to a separatory funnel in which 100 cc chloroform was already present. After cooling, the solution was shaken for 30 minutes. This was repeated twice with fresh chloroform. The 3 fractions of chloroform were mixed and shaken twice with 10 cc 8% NaOH. The NaOH was then made up to 25 cc in a volumetric flask. Two samples of 5 cc each were titrated with bromine. The remaining 15 cc were slightly concentrated by evaporation before a fan and then acidified with concentrated HCl. The precipitate was dried, weighed and titrated.

¹⁰ de Jong, A. W. K., Am. J. Med. Sc., 1933, 185, 630.

¹¹ Greenberg, D. M., and Mackey, M. A., J. Biol. Chem., 1932, 96, 419.

TABLE I.
Contents of Glycuronic Acid and Cinnamic Acid of the Water-soluble Fraction in
the Extraction Flask of Lind Apparatus After Extraction for 20 hr.

the mattheway	1,5		
	Glycuronic acid	Cinnar	nic acid
	Found, mg	Found, mg	Calculated*
1	222	166.4	177.6
2	320	248.6	256
3	261	210	209
4	150.8	114.5	121.3
5	195.7	149	156
6	175.0	133	140

^{*}From monocinnamoylglycuronic acid.

The glycuronic acid content of the 25 cc of watery solution was about equivalent to the amount of cinnamic acid found. When N mg glycuronic acid (M.W. 184) were found by reduction, the cinnamic acid content proved to be about 148/184 X N mg (Table I).

The stoichiometric relation existing between glycuronic acid and cinnamic acid in the fluid analyzed is a strong indication that monocinnamoylglycuronic acid was excreted after administration of cinnamic acid to humans, especially if one takes into consideration that the cinnamic acid compound is water-soluble, insoluble in chloroform, hardly soluble in ether and is easily hydrolyzed by boiling with HCl or NaOH.

6. Absence of cinnamoylglycine. Cinnamoylglycine, being ether soluble, if present should have been found in the ether after extraction in the Lind apparatus. In order to test for the presence of cinnamoylglycine, the crystals obtained after the distillation of the ether were dissolved in boiling water and crystallized. The fraction which crystallized when the solution was still warm was separated and recrystallized from hot water. After purification the crystals did not give the cinnamic acid reaction with Na₂CO₂ and KMnO₄.

Table II shows the excretion of hippuric acid in the four-hour period following the administration of cinnamic acid to humans. Six grams of cinnamic acid (equivalent to 5 g benzoic acid) brought about the excretion of 3.6-5.3 g of hippuric acid. These amounts are practically the same as those following the administration of equivalent amounts of benzoic acid.¹² These results indicate that the oxidation of cinnamic acid to benzoic acid occurs rapidly.

¹² Snapper, I., Klinische Wochenschr., 1924, 3, 56; Quick, A. J., Am. J. Med. Sc., 1933, 185, 630.

TABLE II. Excretion within 4 hr.

	After 5 g benzoic acid	Af	ter 6 g cinnamie	acid
Diagnosis	Hippuric acid	Hippuric acid	Cinnamic acid g	Glycuronic acid
Sp. Reconvalescent Dysentery	5.00	4.31	0.216	0.596
L.S.S. Reconvalescent Diphtheria	4.32	3.60	0.294	0.458
F.L.Y. Healed Kala Azar	4.82	5.25	0.384	0.798
Y.H.S. Reconvalescent Lobar pneumonia	5.07	4.86	0.203	0.653
L.K.S. Reconvalescent Diphtheria	4.77	4.32	0.286	0.632
L.S.C. Avitaminosis A	5.12	4.72	0.202	0.635
C.H.C. Dieneephalic Syndrome	5.89	5.28	0.367	0.654

During the same 4-hour period 400-800 mg of glycuronic acid were excreted. A small part of the cinnamic acid escapes β -oxidation. After giving 6 g of cinnamic acid to persons with normal liver function 200-400 mg of cinnamic acid, conjugated with glycuronic acid, were excreted within 4 hours (Table II).

11347

Splenectomy and Benzol Injection as Means of Increasing Susceptibility of Chinese Hamsters to Kala-Azar.

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From the Department of Medicine, Peiping Union Medical College, Peiping, China.

It has been shown in experimental animals that splenectomy and benzol injection frequently give rise to an increase of susceptibility of the animals to bacterial, parasitic, as well as virus infections.^{1, 2, 3} In parasitic infections such observations have been made with plasmodia, spirochetes and trypanosomes in monkeys, mice, rats, squirrels, etc. The effect of splenectomy and benzol injection on the susceptibility of the Chinese hamster, *Cricetulus griseus*, to *Leishmania donovani* has not been hitherto recorded. The following experiment was, therefore, designed, and the findings are here reported. The flagellate form of *Leishmania* was employed in the present study.

The experiment consisted of 3 groups of 20 hamsters each. The first group consisted of 20 splenectomized hamsters, the second, 20 benzol-injected hamsters and the third, 20 normal hamsters as controls. Splenectomy was carried out under ether anesthesia. All the splenectomized hamsters were allowed to recover from the effects of the operation for a period of from 1 to 2 months, before they were given the inoculation of flagellates. Of the 20 hamsters in the benzol group 10 were each injected subcutaneously with a single dose of 0.5 cc of a mixture of equal parts of benzol and sterile olive oil, 5 with 1 cc each, and another 5 with 2 cc each. The interval between the injection of benzol and inoculation of flagellates was 30 hours in the 0.5 cc group, 48 hours in the 1 cc group, and 7 days in the 2 cc group. From a preliminary determination it was found that the maximum tolerated single dose of the benzol mixture approaches closely to 2 cc for an average hamster. The inoculation of flagellates seems to be preferably given about 48 hours after the benzol injection. The flagellates used in the present study were prepared by pooling the condensation fluid of some 20 tubes of a 13-day culture which had been grown on NNN medium from the spleen emulsion of a heavily infected hamster. On May 10, 1939, each of the 60 hamsters in the 3 groups was given on the same day a single intraperi-

¹ Adler, S., Trans. Roy. Soc. Trop. Mcd. and Hyg., 1930, 24, 75.

² Meleney, H. E., J. Exp. Med., 1928, 48, 65.

³ Zinsser, H., and Castaneda, M. R., J. Exp. Med., 1930, 52, 649.

toneal inoculation of 0.2 cc of the culture, estimated to contain 145,000 flagellates.4

After the inoculation of flagellates infection in the hamsters was first determined by liver puncture and then by the examination of smears and sections made from the spleen and liver at autopsy. Two liver punctures were done before the hamsters were sacrificed for examination. Only 3 or 4 hamsters from each group were punctured. The first puncture was done 29 days and the second, 56 days after the infective inoculation. The results of the first puncture were all negative. But by the time of the second puncture 1 or 2 hamsters from each group already showed positive smears. order to make use of the rate of early infection to determine the degree of susceptibility, all the hamsters which had survived up to the time of the second puncture were sacrificed. Not only were smears and sections made from the spleen and liver of the hamsters but also the weight of the hamsters as well as that of their spleen and liver were recorded. During the period of infection 2 hamsters from the splenectomized group died. Their organs were not suitable for examination because of marked post-mortem changes. The results of the examination of the spleen and liver of all the remaining hamsters are shown in Table I in which only the parasitological findings of sections were recorded, as they were found to be more conclusive than those afforded by direct examination of smears.

As shown in Table I, in the group of 18 splenectomized hamsters which were killed and examined at the conclusion of the experiment all except one showed a large number of parasites in the sections made from the liver. On the other hand, in the group of control hamsters, only 8 out of 18 (liver of 2 hamsters not examined) hamsters gave positive liver sections, in most of which only a few parasites were found, although positive spleen sections were seen in 14 of the 20 hamsters. In this group 6 hamsters failed to take the infection. In the benzol-injected group 4 hamsters showed negative spleen sections and 9 showed negative liver sections. Four hamsters in this group failed to contract kala-azar. There was no striking difference of the infection rate among the various dosage groups of benzol-injected hamsters. The 2 cc group seemed to have given the highest infection rate, but repeated smaller doses which were not tried in the present study, might give a higher infection rate.

Table II shows the average weight of the hamsters and that of their spleen and liver. In the benzol group the weighing of the

⁴ Earle, W. C., and Perez, M., J. Lab. and Clin. Mcd., 1932, 17, 1124.

	Hamsters.	
	Normal	
	and	
	Benzol-in jected	
TABLE I.	ver of Splenectomized.	,
	nd Th	
	e Spilon n	2
	Castions	Deer Louis of
	The state of the s	reastrological ringings or
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		e l	Benzol-injected hamstors	nstora		Control hamsters	78 (
ď	Splenectomized namsters		Leishman-Donovan	-Donovan		Leishman-Donovan	-Donovan
Hamster No.	Leishman-Donovan bodies in liver sections	Hamster No.	bodies in sections of liver	ections of spleen	Hamster No.	liver sections or liver	apleen
3003 3004 3005 3005 3007 3008 3008 3010 3011 3014 3014 3016 3016 3018 3018 3018 3018	died (not examined) (+++++++++++++++++++++++++++++++++++	3035 3035 3036 3038 3039 3041 3041 3044 3073 3073 3073 3073 3065 3065 3065 3065	+ ++ ++ ++ (+ (+ ++++++++++++++++++++++	+++++ + + + + + ++++++	3045 3046 3046 3048 3050 3050 3051 3081 3082 3083 3083 3083 3083 3083 3083 3083	÷ + + + + + + + + + + + + + + + + + + +	+ + + + + + + + + + + + + + + + + + +
Hamste ",		ceived 0.5 ec of '' 1.0 '' '' 2.0 '' ''	incl. each received 0.5 ec of benzol mixture.				
10+++	Not examined Not found Very few found Found in every 11-20 oil in '' '' 6-10 '' '' '' 1-5 ''	11-20 oil immersion fields. 6-10 ''' ''' ''' 1-5 ''' '''					

TABLE II.

Average Weight of Body, Spleen and Liver of Splencetomized, Benzol-injected and Normal Hamsters.

	Body weight, g	Liver weight, g	Spleen weight, g
Control hamsters	26.1	1.023	0.089
Splenectomized hamsters	28.7	1.320	
Benzol-injected hamsters	26.6	~~	0.105

liver was not done. Taking the normal weight of the hamster's spleen as from 0.15 to 0.3% of the body weight it is evident that the average weight of the spleen in both benzol-injected and control hamsters was above normal. The increase of the spleen weight was apparently due to the kala-azar infection. The difference of the average spleen weight between these two groups seems to be out of proportion to the difference of their average body weight. As the rate of spleen infection was higher in the benzol-injected hamsters than in the controls, it appears likely that the greater average spleen weight of the benzol-injected group was due to the heavier infection rather than the greater average body weight. In the splenectomized hamsters the average liver weight was again greater than that of the controls. This was also likely due to the heavier infection rather than the greater average body weight of the splenectomized animals, although a part of the increased liver weight might have been due to a compensatory hyperplasia of the reticulo-endothelial system.

Conclusions. Chinese hamsters became more susceptible to infection with the flagellates of Leishmania donovani after splenectomy or benzol injection. Of the two means employed to increase the susceptibility of hamsters to kala-azar infection splenectomy appears to

be more effective.

⁵ Melency, H. E., Am. J. Path., 1925, 1, 147.

11348 P

Optic Nerve Response to Retinal Stimulation in the Rabbit.*

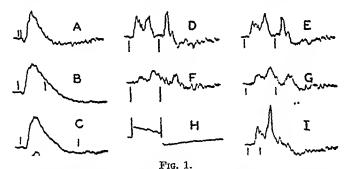
S. HOWARD BARTLEY AND GEORGE H. BISHOP.

From the School of Medicine, Washington University, St. Louis, Mo.

With changes in intensity and duration of light, the retinal potential undergoes slight changes in form, while the nerve discharges show transformations corresponding to the form of the stimulus.

The fore part of the rabbit's brain was removed under ether, exposing the optic nerves. Records were taken from one nerve and from across the corresponding retina. Light intensities used were high but within the physiological range, as indicated by reduction of response with reduction of intensity. Experiments were conducted in a dark room, flashes being delivered at about 1 per second from a 2-mm slit in the lamp housing, in front of which a sector disk was rotated. A lens projected an image of the slit on the rabbit's cornea, the eye thus focused an image of the lens on the retina. This image stimulus was compared with one from a diffusing screen close to the cornea illuminated with a 1½-inch spot of light.

With flashes as short as 5 ms the retinal potential shows the usual a wave, a diphasic b wave, and no c. The "off" effect does not appear. With longer durations the b wave assumes its conventional



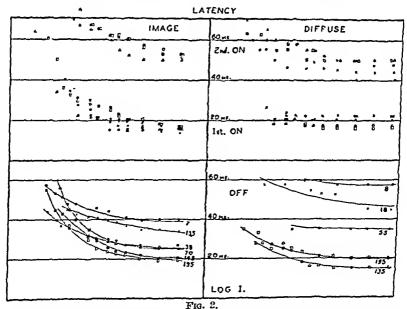
A, B, C, records from retina, light 13,000 candles per sq ft on 10 sq mm retinal image, durations of 7, 85, and 195 ms respectively, as marked. D.C. amplifier. Oculomotor nucleus destroyed after A. D, E, F, records from optic nerve, same stimulus, 101 ms duration, but with apertures at lens of 72, 30, and 8 mm diameter, ratios of 1, 1/6, and 1/81 areas of image. G, 72 mm aperture, intensity 1,800 candles per sq ft, or 1/10th the previous. This record falls between 1/6th and 1/81st aperture records in amplitude. H, photocell record. I, like D, shorter duration, 40 ms. The "off" spike is summed with the second "on" wave.

^{*} Assisted by grants from the Rockefeller Foundation and from the Scottish Rite Masons' Fund.

monophasic form which does not return to the base line during illumination. (Fig. 1.) Between 5 and 200 ms duration, and between 18,000 and 200 candles per sq ft, the form of the retinal b wave changes surprisingly little.

On the contrary, the nerve discharge alters progressively over these ranges. The characteristic "on" response to a short bright flash occurs in two parts, the first consisting of a sharp initial spike followed usually by a decreasing series at 10-ms intervals, and the second, a rise which falls off during further illumination, its peak at about 30 ms after the first. The "off" response consists of a spike series similar to the first "on".

As duration is decreased, the "off" response decreases in amplitude, but is still distinct at 7 ms, while the "on" discharges decrease only at still shorter durations. As intensity is decreased, the "on" responses decrease in amplitude more rapidly than the "off", and the first "on" discharge more rapidly than the second, suggesting that different fibers are involved. The second "on" response and the "off" may sum quantitatively, again indicating different fibers in



Latency of response vs. log. intensity for the three prominent waves of optic nerve. Left, 10 sq mm image on retina; right, diffusing screen at cornea, nearly whole retina illuminated. Range of intensity, 18,000 to 500 candles per sq ft. Stimulus duration in milliseconds at right.

these two responses. With decrease of either intensity or duration, the latencies of all responses increase. (Fig. 2.)

We infer from such records that the retinal activity arises in elements distal to the ganglion cell layer, and probably in the sense cells; and that the total b wave represents the summation of impulses which are individually briefer. Records obtained with and without a diffusing screen are so closely similar as to indicate that the responses observed following projection of a small bright image on the retina are chiefly due to stray light, illuminating the retina as a whole by internal dispersion from the image.

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Influence of Aldehydes on Transplanted Tumors.

CHRISTOPHER CARRUTHERS. (Introduced by E. V. Cowdry.)

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St. Louis, Mo.

Strong obtained retrogressive changes and liquefaction of spontaneous tumors in mice^{1, 2} and in dogs³ by heptaldehyde. He also observed that the low boiling fraction of the oil of gaultheria was more effective than heptaldehyde alone,⁴ an effect probably due to the presence of naturally occurring antioxidants which prevent the autooxidation of heptaldehyde. Boyland and Mawson⁵ were able to induce some inhibition of both grafted and spontaneous tumors with citral, but heptaldehyde only inhibited the latter.

On the other hand, Baumann, Kline, and Rusch⁶ were unable to influence a spontaneous mammary adenocarcinoma, or tumors induced by ultraviolet light, or by benzpyrene by adding heptaldehyde to the diet of mice. Clarke⁷ found that heptaldehyde had no significant effect upon a transplanted spindle cell sarcoma in 14 rats. Orr and Strichland⁸ were likewise unable to affect spontaneous and trans-

¹ Strong, L. C., Am. J. Cancer, 1939, 35, 401.

² Strong, L. C., Science, 1938, 87, 144.

³ Strong, L. C., and Whitney, L. F., Science, 1938, 88, 111.

⁴ Strong, L. C., Yale J. Biol. and Med., 1938-39, 11, 207.

Boyland, E., and Mawson, E. H., Biochem. J., 1938, 32, 1982.
 Baumann, C. A., Kline, B. E., and Rusch, H. P., Proc. Soc. Exp. Biol. and Med., 1938, 39, 354.

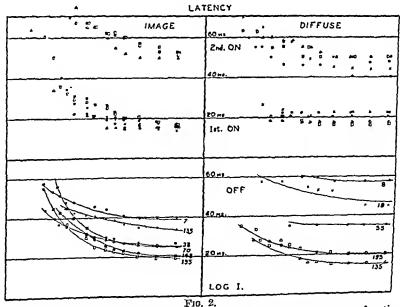
⁷ Clark, W. G., PROC. Soc. EXP. BIOL. AND MED., 1939, 40, 562.

⁸ Orr, J. W., and Strichland, L. H., Yorkshire Council of the British Empire Cancer Campaign, 1938-39, 8.

monophasic form which does not return to the base line during illumination. (Fig. 1.) Between 5 and 200 ms duration, and between 18,000 and 200 candles per sq ft, the form of the retinal b wave changes surprisingly little.

On the contrary, the nerve discharge alters progressively over these ranges. The characteristic "on" response to a short bright flash occurs in two parts, the first consisting of a sharp initial spike followed usually by a decreasing series at 10-ms intervals, and the second, a rise which falls off during further illumination, its peak at about 30 ms after the first. The "off" response consists of a spike series similar to the first "on".

As duration is decreased, the "off" response decreases in amplitude, but is still distinct at 7 ms, while the "on" discharges decrease only at still shorter durations. As intensity is decreased, the "on" responses decrease in amplitude more rapidly than the "off", and the first "on" discharge more rapidly than the second, suggesting that different fibers are involved. The second "on" response and the "off" may sum quantitatively, again indicating different fibers in



Latency of response vs. log. intensity for the three prominent waves of optic nerve. Left, 10 sq mm image on retina; right, diffusing screen at cornea, nearly whole retina illuminated. Range of intensity, 18,000 to 500 candles per sq ft. Stimulus duration in milliseconds at right.

TABLE I. Effect of Oral Administration of Heptaldehyde and of Polargonic Aldehyde on the Marsel Buffalo Adenocarelnoma. Product of averago tumor dimonsions (L X W) in em at days after treatment was started.

						Days										
No. of mice	0		2			10			15			ន្ព			윉	
C II D	1	0	1	4	O	H	P	0	1	!	D	1	{	O	H	4
	ჟ	1,3		9	1.9	1.3	5.0	3,0			3.6			4,4	e E	
40	7. 8. 6.	1.4	1,6	1,8	5.5	ci 2	3,4	3.6	3,8	4.9	6,4	5,8	0.0	6.3	6.4	
	1,3	6,3	- 1	4.4	3.7	3.5	3.4	4.4			5.7	- 1		6.3	6.3	
,		ì	! !													!
	\$ P	•		•	TABLE II.	E II		•		-						
	Encet of the intriperitonen injection of itablanchy of the A. Carcinoma,	o ruceat	GLICON		Jection	10	obtando	o onto	200	2 2	inonia,					
	Sum of averago tumor dimensions (L + W) in em at days after treatment was started	mor dir	nensio	us (L	# +	ii.	em at d	ays at	fter t	reatmen	t was a	tarte	-i			
						Days	4									
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C H E	(L + W) cm	Ö	Н	2	ರ	=	討	Ö	H	11	೮	Η	2	೮	H	×
0 4 0	T.N.P.					1.1						; ;			0:3	
6 10 4	T.P.	1.7	1,1	1,7	с1 Ф	1.8	2.0	33	2.7	3,8	1,7	3,9	8,4	8,4	8,4	1.0 8
1 0	.0-1.0		1,8		3,4	င်း ထ		3.9			10	Ť,		8	5.0	
0 0 1	1-2.0		63	63			3.1			3,9			4.7			9.9
0 1 0	2-3.0		3.7			4.3			5,3			6,1			6.1	
C-Control; II-	G-Control; II-Heptnidehyde; P-Pelargonic Aldehyde; E-Billyl Bsters of Lard; T.N.PTumors not Palpable; T.PTumors	elargonie	Alde	hyde;	E-E	thy!	Ssters o	f Lar	I; T.	N.P.—7	umors	not I	alpable	T.P.	Tm	nors

planted tumors in mice, guinea pigs and in a dog by oral administration or injection of heptaldehyde.

Experiments were therefore undertaken to study the effect of aldehydes, especially heptaldehyde and some of its compounds, on transplanted carcinomas.

Oral administration of pclargonic aldchyde and of heptaldehyde.* Emulsion from one tumor was injected at the same time into enough mice to serve for the experimental groups and for controls. food was Purina Chow. As soon as tumors became palpable, the mice were divided into groups of comparable tumor diameters. One group received pelargonic aldehyde, one received heptaldehyde, while the other served as controls. Forty to fifty mg of the aldehyde were administered orally every day until the experiment was terminated. The product of the maximum length and width (L X W), was used as a criterion for the growth rate of the tumors. results of this experiment are indicated in Table I. The products of the tumor dimensions are recorded every 5 days and the increase in size can be observed as one reads the table from left to right. The first column shows the number of mice for the different groups with letters to indicate the type of treatment. The product of the tumor dimensions for any 5-day period for any of the 3 particular groups can be found by reading under the letter in question. Since Strong's outstanding results were with small tumors, in this and in the following experiments, treatment was begun when tumors were either not palpable, or were very small, in order to study the effect of aldehydes upon tumors before they became so large that any inhibitory effect might not be indicated. Hence, there are as many horizontal columns as there are tumors grouped according to initial tumor dimensions. There is no significant inhibition on the growth rate of the transplanted Marsch Buffalo Adenocarcinoma even when the aldehydes were administered to mice bearing small tumors.

To check the possibility that oral administration of heptaldehyde might have an effect on a transplantable tumor not manifested by changes in the growth rate, portions of a tumor were removed from a mouse which had received heptaldehyde (40-50 mg) daily for 27 days, and the tumor suspension was inoculated into 8 females. Since tumors developed in all the mice and grew rapidly, it is evident that the ability of the tumor to "take" was not lost.

To determine whether heptaldehyde given before transplantation would have any effect upon the subsequent growth of the Marsch Buffalo Adenocarcinoma, 20 mice were fed 40-50 mg of the alde-

^{*} From Eastman, redistilled weekly and stored at 0-5°C.

Effect of Intraperitoneal Injection of N-heptaldoxime on A Carcinoma and on Marsch Buffalo Adenocarcinoma. Sum of average tumor dimensions (L + W) in cm at days after treatment was started. TABLE III.

				Days	8									
No. of mice	0		10	10			15			20			25	
	Tuitial termon			A Carcinoma	ma.									
24	(L + W) cm		E	Ö	臼	٥	æ	岡	ပ	æ	闰	Ö	æ	Þ
1 1 0	T.P.	1.7	1.3	1.5 2.	2.0	2,1	8.3		2,5	3,1				
10	1-2.0		2.2 2.4	3.3	9 2.9	3.5	3.5	3.4	4. 3.	3.9	3.8			
Н	2.1-3		3.7 3.4	4.	5 4.1		5.2	4.6		1	6'₹			
			sch	Buffalo Ade	mocarcino	ma.								
	T.P.		9.	H	~		2.6							
5 6 4	0.1.0	1.9	2.0	2.9 3.5	3.0	3.7		3.8	3.7	4.1	4.0	4,3	4,3 4,4 4.4	4.4
	TABLE IV.	Š	,	TABLE I	٧.	;								
	Effect of the	Bisultite	Addition	1 Compound	of Hepts	ildehyde	T IIO	Carein	oma.					1
				Даув	F.									
No. of mice	0		5	10			15			8			25	ſ
C Bi	Initial tumor $(L + W)$ cm	٥	R1	Ü	128	٥	R		O	l m	=	٥	l æ	-
	T.N.P.		1.0		1.8		2.7			খ	 4		44	6
	G.F.	1.7	1.8 6	8:3	87.0	63	3.55		4.7	4.9	9	4.5	5.2	C3
	0-T-0	2.2	0 10		3.2	3.9	4.0		5.3	ທ່	~	5. 8.	က်	œ
T	0.5-1.1		7.5		4.3		0.9			ő	∞		÷	0

C-Control; R-N-Heptaldoxime; E-Ethyl Esters of Lard; R1-NaHSO3-Addition Compound; T.N.P.-Tumors Not Palpable; T.P.-Tumors Palpable,

hyde orally every other day for 19 days prior to inoculation of the tumor, and up to the time the experiment was terminated (25 days). Seven mice served as controls. Nineteen tumors occurred in the experimental group and in all of the controls, and the subsequent growth rate of the tumors in both groups was not significantly different.

Intraperitoneal injection of heptaldehyde. It has been observed that heptaldehyde is capable of eausing resorption of mouse embryos, and is especially effective when dissolved in the ethyl esters of lard and injected intraperitoneally." On the assumption that the amount of aldehyde reaching the tumor after oral administration is less than after intraperitoneal injection, an attempt was made to inhibit the growth of transplanted tumors by intraperitoneal injection. Twenty-eight strain A mice were inoculated with an emulsion obtained from a spontaneous mammary earcinoma of the same strain, and as soon as tumors became palpable in some of the mice, heptaldehyde was injected intraperitoneally in graded doses because of its toxicity every day into 16 mice: For 3 days .03 cc of aldehyde in .06 ec ethyl esters of lard, for 2 days .045 ec of the aldehyde in .075 cc ethyl esters of lard, for 8 days .06 cc of the aldehyde in .06 cc ethyl esters of lard, for 13 days .09 cc of the aldehyde in .075 cc ethyl esters of lard.

Five mice were likewise treated with the ethyl esters of lard without aldehyde, while 8 served as untreated controls.

The sum of the maximum length and width (L+W) in cm was used as a criterion for the growth rate. Since the treatment was started as soon as a few tumors became palpable (in order to study the effect of heptaldehyde on the growth of tumors not palpable when the treatment was started), the initial tumor size for 3 groups varied. The results given in Table II show that heptaldehyde did not inhibit the growth rate of this carcinoma when the initial tumors were small (T.P.), and, also, that it was ineffective even when the tumors were not palpable (T.N.P.) when the injections were started. Similar results were obtained for the transplanted Marsch Buffalo Adenocarcinoma in 9 mice in which the tumors were either not palpable or were very small when the treatment was started.

Intraperitoneal injection of n-heptaldoxime. N-heptaldoxime (Eastman) was dissolved in the ethyl esters of lard (.05 cc contained 2 or 4 mg of the oxime) and its effect upon the 2 transplanted tumors was studied. As soon as tumors became palpable after transplantation, each mouse received 2 mg of the oxime per day for 2 days, then

⁹ Carruthers, C., Proc. Soc. Exp. Biol. and Med., 1939, 41, 336.

complicating factor as young mice were used as hosts for transplanted tumors. Bischoff and Long¹¹ have shown that sarcoma 180 grew to a greater size in young Marsch Buffalo mice than in older mice. This strain of mice also develops spontaneous mammary carcinoma.

On the other hand, Strong's results are obtained over a long period of time and in most of his experiments the aldehyde was mixed with the diet. This procedure might allow the proöxidant, heptaldehyde, by initiating autoöxidation or by the formation of peroxides, to induce dietary changes and thus influence tumors indirectly. The odor of heptaldehyde disappears quite rapidly when mixed with diets at room temperature.

Experiments are in progress to determine whether or not heptaldehyde will inhibit the growth of transplanted tumors in mice whose vitamin E stores have been depleted, since the tocopherols are known antioxidants¹² which may inhibit, at least partially, the action of the proöxidant, heptaldehyde.

Summary. Under the conditions of these experiments, the growth rates of the transplanted Marsch Buffalo Adenocarcinoma, of the transplanted A carcinoma were not significantly altered when: (1) heptaldehyde and n-heptaldoxime were dissolved in the ethyl esters of lard and injected intraperitoneally; (2) the bisulfite addition compound of heptaldehyde was injected subcutaneously. The oral administration of heptaldehyde and of pelargonic aldehyde was also without influence on the progress of the transplanted Marsch Buffalo Adenocarcinoma.

¹¹ Bischoff, F., and Long, M. L., Am. J. Cancer, 1936, 27, 104.

¹² Olcott, H. S., and Emerson, O. H., J. Am. Chem. Soc., 1937, 59, 1008.

4 mg per day until the experiment was terminated. One group of mice served as controls, while another received injections of the ethyl esters of lard. Evidently (Table III), n-heptaldoxime had no significant effect upon these 2 transplanted tumors even when treatment was started when they were small (T.P. and [L+W] of O-2.0).

Subcutaneous injection of the bisulfite addition compound of heptaldehyde. Heptaldehyde was added to a saturated solution of NaHSO3, the addition compound was thoroughly washed with distilled water, and dried over CaCl₂. The addition compound was made weekly and dissolved in distilled water prior to subcutaneous injection. In a preliminary experiment 5 mg of the addition compound were injected daily into 6 strain A mice bearing the A carcinoma, but no inhibitory effect was noticed, although the tumors were small when the treatment was started. Later 20 mg of the compound were injected daily into the same strain bearing the same carcinoma, but the results (Table IV) give little indication of any inhibition even when the initial tumor (L+W) is small (T.P.), or even when tumors could not be palpated when treatment was started (T.N.P.). Nine mice bearing the transplanted Marsch Buffalo Adenocarcinoma were also treated with 20 mg of the addition compound daily, with the same results. Mice bearing both of these tumors were also treated with an equivalent amount of NaHSO3, but without effect.

None of the above compounds induced noticeable liquefaction of the tumors. Subcutaneous injection of heptaldehyde resulted in necrosis which prevented this type of administration. Heptaldehyde ammonia had a similar effect and it was highly toxic via the intraperitoneal route.

Strong's success with heptaldehyde was with spontaneous tumors. Practically no effect has been demonstrated on transplanted tumors. The reason for this difference is unknown. However, Cramer and Horning¹⁰ have shown that "brown degeneration" occurs in the adrenals, at least in several strains of mice which have a high incidence of spontaneous mammary carcinoma. Since spontaneous tumors arise in older females, the question arises as to the relative efficacy of heptaldehyde in young and in old mice bearing tumors, especially in the latter, because "brown degeneration" of the adrenals may be indicative of other endocrine changes, thus possibly allowing greater aldehyde activity. "Brown degeneration" has been observed in the adrenals of strain A mice in this laboratory, but was not a

¹⁰ Cramer, W., and Horning, E. S., Lancet, 1939, 192.

smears; those of the males by direct tests for copulation. The mean age of first estrus for the 82 females was 51.1 days; the mean age at first copulation by the males was 58.3 days. The extremely early and extremely late cases were used as the parental generation.

The sire used from the parental generation representing the early strain copulated at the age of 44 days, and the 11 dams of the early strain were first in estrus at ages ranging from 35 to 43 days (mean, 40.0 days). For the late strain there were 2 sires, one copulating at the age of 70 and the other at the age of 76 days, and 10 dams with ages of first estrus ranging between 58 and 73 days (mean, 65.3 days). In the 6 succeeding generations the early pubescent animals selected for breeding never exceeded the puberal age of 49 days; the means for males and for females ranged between 37 and 43 days, with that of the males always slightly lower. The puberal age of males and females representing the late strain never fell below 55 days. For the late males the means ranged between 70 and 75 days and for females between 62 and 65 days.

The cumulative effects of selective breeding on age of puberty shown by the Fo generation is given in Table I and is graphically

TABLE I.
Frequency Distributions of Ages of Puberty in Albino Rats of the 6th Selectively
Bred Generation.

	177.0	d Generation	/11,	
Ages	Early Males	Early Females	Late Males	Late Females
34-36	19	11	1	0
37-39	16	21	0	1
40-42	20	31	1	1
43-45	23	26	2	1 8
46-48	29	26	2	12
49-51	18	10	1 2 2 6 9	7
52-54	11	1	9	12
55-57	8 6	1 1	10	14
58-60	6	0	10	8
61-63	6 3	0	9	8 6 2 6 5 2 1
64-66	3	0	7	2
67-69	0	0	7	6
70-72	2	1	9 1 1 2 1	5
73-75	0	0	1	2
76-78	0	0	1	1
79-81	3 0	0	2	1
82-84	0	в	1	1
85-87	0	0	0	2
88-90	0	0	1	0
91-93	0	0	0	0
94-96	1	0	0	0
97-99	1	0	1	0
		~		
Total	166	128	80	89
Mean	47.56	42.98	61.18	56.87
σ	10.56	5,07	10.47	10.38

11350

Change of the Age of Puberty in Albino Rats by Selective Mating.*

CALVIN P. STONE AND R. G. BARKER.

From the Department of Psychology, Stanford University.

During 3 decades, ending about 1922, the reported age of puberty in albino rats shifted downward approximately 20 to 30 days. This change has been ascribed in part to improved diets and husbandry, in part to the use of criteria based on estrus and copulation, rather than on fecundation, and in part to other still unrecognized causes. Among the latter, selective breeding has long been suspected of playing an influential rôle in view of the fact that animal breeders are not able to separate accurately the genetically late pubescent animals from those actually retarded because of illness, malnutrition, over crowding, or other adventitious circumstances contributing to what they consider undesirable breeding stock. A confusion of this kind would, in time, reduce the proportion of genetically late maturing animals in the breeding colony and thus tend to lower the mean ages of puberty in representative animals from the colony. To throw some light on the readiness with which selective breeding might affect mean age of pubescence, the present experiment was under-

In starting the experiment, 25 pairs of albino rats, descendants of the inbred Slonaker colony and originating approximately 35 years ago from Wistar stock, 6 months of age and known to be fertile were mated and allowed to produce one litter each; from these the parent generation was selected. Although we realized that a still larger number of pairs was desirable in order to provide a goodly number of extreme cases, facilities at our disposal at that time prevented our exceeding the number specified above. Nothing was known concerning the ages of pubescence of these 25 pairs.

From the 25 litters 62 males and 82 females were reared. So far as possible living conditions for all of the litters were kept similar and a regular routine of feeding, handling, and examining the young was adopted so as to minimize variables in this sphere of influence. All of this generation and those which followed were fed the Steenbock mixture, supplemented with lettuce once a week. The ages of pubescence of the females were determined from vaginal

^{*}This study was financially supported by a grant from the Committee for Research on Sex Problems, National Research Council.

A 11351 P

Bacteriostatic Action of Various Wetting Agents upon Growth of Tubercle Bacilli in vitro.*

B. L. FREEDLANDER. (Introduced by Ernest L. Walker.)

From the Department of Medicine, University of California Medical School, the San Francisco Hospital, and San Francisco Department of Public Health.

Wetting agents in general possess the properties of penetrating and emulsifying surface films of fatty substances to a high degree, and are powerful surface tension reducers. For these reasons, it is of interest to observe their action on tubercle bacilli with their enveloping fatty capsule. The bactericidal action of several wetting agents on some organisms have been investigated, but little has been done in testing the effect of these compounds on the growth of tubercle bacilli.

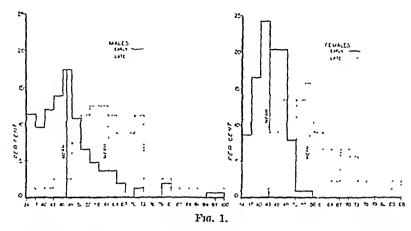
The following compounds were tested for bacteriostatic action: Nacconol NR,† an alkyl aryl sulfonate; Aerosol OT-100, an ester of a sulfonated bi-carboxylic acid; Aerosol OS, a sodium salt of an alkyl naphthalene sulfonic acid; and Zephiran, a mixture of high molecular alkyl-dimethyl-benzyl-ammonium chlorides. All the agents were approximately neutral in solution.

A non-virulent Novy strain of tubercle bacilli was grown on liquid synthetic asparagin media containing 6% glycerin, with pH 7.4. This produces a heavy surface growth in 2 to 3 days. Dilutions of the wetting agents were made in normal saline and added in equal amounts to tubes containing 4.5 cc of media. A large loopful of surface growth of a 3-day culture of tubercle bacilli was inoculated on a cork float in each tube. The tubes were then slanted and incubated at 37°C. Readings of the amount of growth were made at 2-day intervals.

The results are shown in Table I. The most effective wetting agent is Zephiran, which produced complete inhibition of growth in dilution of 1:80,000, and retardation of growth in dilutions as high as 1:400,000. Nacconol NR and Aerosol OT-100 prevented growth in dilution of 1:5,000, and retarded growth in dilution of 1:40,000. Aerosol OS produced the lowest bacteriostatic action,

^{*} This investigation was aided by a grant from the San Francisco Tuberculosis Association.

[†] Nacconol, a product of the National and Chem. Co.; Aerosol, a product of the Amer. Cyanamid & Chem. Corp.; Zephiran, a product of the Alba Pharmaceutical Co.



represented in Fig. 1. The mean ages of puberty for early and late males of the F₆ generation differ by 13.6 days; those for females by 13.9 days. For each sex the difference in means is statistically significant.

As the distributions clearly show, there is still a large amount of overlapping in the two strains. Further selective breeding should reduce this amount, but it is unlikely that it will eliminate it entirely. Two reasons for this may be given. Mild cases of illness and malnutrition tend to retard a few animals of the early strain in each generation and thus contribute to overlapping. Also the experimenter is unable to differentiate with the required exactitude those animals that are genetically late in maturing from those that are late because of both the genetic factor and adventitious factors that cause retardation. Thus he is not able to obtain in every instance the very best breeders to represent the late strain.

Summary. In this paper evidence is given which clearly indicates the possibility that lowering of the mean age of puberty in rat colonies during the past 30 years may have been due, in part, to selective breeding of early pubescent stock.

permitting growth in dilution of 1:1,000, and retarding growth slightly in dilution of 1:10,000. The above results are not strictly comparable, as the degree of chemical purity of each of the compounds is unknown.

Although the bacteriostatic action of Zephiran against tubercle bacilli is high, its bactericidal action is low. To 4.5 cc of a 1:1000 dilution of Zephiran was added ½ cc of an undiluted 3-day growth of tubercle bacilli, which previously had been well shaken to break the surface film into small particles. This mixture was incubated at 37°C for ½ hour, then centrifugalized, washed twice with normal saline, and the sediment planted on Lowenstein's egg media. The growth was positive.

The superior bacteriostatic action of Zephiran over that of the other wetting agents is not due entirely to its ability to reduce surface tension or to superior wetting qualities, as these qualities are approximately the same in all the compounds tested. Its superior action is probably inherent in its molecular structure.

11352 P

Functional Transplants of Epithelial Hypophysis in Three Species of Amblystoma.

WAYNE J. ATWELL AND JAMES W. TAFT. From the Department of Anatomy, University of Buffalo.

Previously it has been shown^{1, 2} that transplantation of the primordium of the epithelial hypophysis, independent of brain and foregut, may be followed by differentiation and function of the transplant in 2 species of frogs, Rana sylvatica and R. pipiens. Although numerous transplants were attempted with embryos of Amblystoma punctatum no unequivocally successful cases were obtained. This seemed to confirm the conclusions previously reached by Blount,³ who held that the epithelial portion of the hypophysis is dependent upon an association with the neural lobe for its differentiation and functioning.

More recently Blount4 has presented evidence to show that hypo-

¹ Atwell, W. J., PROC. Soc. EXP. BIOL. AND MED., 1935, 33, 224.

² Atwell, W. J., Anat. Rec., 1937, 68, 431.

Blount, R. F., J. Exp. Zoöl., 1932, 63, 113.
 Blount, R. F., Anat. Rec., 1939, 73, Sup. 1, 7.

Action of Four Wetting Agents on Growth of Tubercle Bacilli in vitro.

			TO THE OUT WELLING A	GEI
	AprogolOS	2 4 6	25+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+ 4+	
Growth	Acrosol OT-100	2 4 6 days days	1 1 1 1 1 1 1 1 1 1	
Gri	Nacconol	days days days		
	phir	2 4 6 days days days		78; 4+ in 4 days.
		Dilution	115,000 115,000 115,000 1160,000 1160,000 1160,000 1170,000 1180,000 11155,000	Controls: 3+ in 2 days; 4+ in 4 days.

lobe or a distinct pars tuberalis be recognized. Some of the transplants consisted in part of epithelial cysts or vesicles, the derivation of which is not always clear. Some of them, however, exhibited the structure of the epidermis. A few may have been derived from cells inadvertently removed from the brain floor and included with the transplant.

The successfully transplanted cases gave evidence of function of the pars intermedia by being dark in color in striking contrast to the silvery condition of hypophysectomized animals without transplants. The dark color was observed to be due to dispersion of pigment in both dermal and epidermal melanophores. Several animals with transplants exhibited a more intense pigmentation than normal, unoperated controls.

In a number of cases the living animals showed a spot of still darker coloration, 34 mm in diameter, in the dorsal skin, caudal to the right eye. In a few instances this dark spot was located in the roof of the mouth or pharynx ventral to the internal ear. In each of the above cases study of serial sections revealed that the transplant was located in relation to the center of the dark area, being either subepidermal or submucosal. This was taken to indicate that in certain cases at least the transplant may exert a local as well as a systemic effect on pigment cells.

The function of the pars anterior of the transplanted hypophysis was evidenced by the normal size of the thyroid glands as contrasted with the atrophic condition found in hypophysectomized animals without transplants. None of the animals had completed metamorphosis although several had made noticeable progress in that direction

Summary. Autoplastic, heterotopic transplants of the primordium of the epithelial hypophysis have been made in three species of Amblystoma. These transplants differentiated without contact with the brain, and gave evidence of chromatophorotropic and thyreotropic activity.

physeal transplants in A. punctatum may produce thyreotropic hormone. Regarding the pars intermedia, however, he states that this lobe "does seem dependent upon the pituitary floor of the diencephalon for its development." Burch, by operations performed on Hyla regilla in the gastrula stage, was able to suppress the differentiation of the pars intermedia and thus to produce silvery animals. Eakin undertook experiments upon Triturus torosus, to confirm Burch's findings. At a stage corresponding to Stage 36 in Harrison's series gelatin was injected into the fore-gut. Following this procedure a single case of 'albinism' was found. This, together with the histological picture, was taken to indicate that the differentiation of the epithelial hypophysis is dependent upon the inducing power of the infundibulum.

The present experiments were performed upon embryos of A. jeffersonianum, A. tigrinum, and of the white axolotl (partial-albino strain of A. mexicanum). Operations were done at the tail-bud stage, corresponding approximately to Harrison's stages 30-32 for A. punctatum. Using care not to remove any of the brain floor the solid primordium of the epithelial hypophysis with some of the associated ectoderm was transplanted in the same animal to a position between the right otocyst and the hind brain. A small incision through the epidermis permitted the insertion of the transplant. The point of entrance was dorsal and rostral to the otocyst. Animals were reared in the laboratory for from 2 to 4 months. Completely hypophysectomized animals without transplants and normal, unoperated salamanders served as controls.

For a transplantation experiment to be considered successful it was required that study of the animal and of the serial sections show: (1) entire absence of the epithelial hypophysis from the orthotopic position, (2) characteristic hypophyseal tissue in the region into which the transplant had been placed, and (3) some evidence of function of the transplant as described below. Forty such 'successful' cases are included in this report, 35 being of A. jeffersonianum, 3 of A. tigrimum, and 2 of the white axolotl. These 40 cases are from 72 operated animals studied at autopsy.

Most of the transplants were contained in the cartilage surrounding the internal ear. A few were dorsal, ventral, rostral or medial to the otic capsule. Only 2 were contained in the cranial cavity. Histologically the transplants showed the structure of the pars intermedia and/or of the pars anterior. In no case could a neural

⁵ Burch, A. B., Proc. Soc. Exp. Biol. and Med., 1938, 38, 608.

⁶ Eakin, R. M., Growth, 1939, 3, 373.

daily intake of approximately 120 mg and equal to at least 500 mg

per kilo of body weight.

The sulfanilamide had no apparent effect on the Bartonella muris. All 15 of the treated rats gave positive blood smears, taken on the third to fifth day after being infected, and 10 died within 12 days. These were practically the same results as obtained in 15 untreated controls where 14 gave positive blood smears and 8 died. The effectiveness of sulfanilamide was further tested by adding 1% to the solution of physiological saline and sodium citrate just described for Group A. The donor blood was added to this 1% mixture and allowed to stand 10 minutes, then injected intraabdominally into the splenectomized recipient rats. The results again failed to show any beneficial effect from sulfanilamide; 13 of the 15 rats in this group showed positive blood smears and all were dead within 12 days The toxicity of the sulfanilamide may have contributed to the high mortality in this group as compared to the controls. In all groups the Bartonella muris bodies disappeared rapidly from the blood and were usually not found after the fifth day, even though the symptoms were so severe that the rats continued in an emaciated condition for several more days. After the twelfth day, few died.2,2

Summary. Splenectomized rats infected with Bartonella muris were treated with sulfanilamide in doses of 500 mg per kilo of body weight. A study of 30 rats thus treated showed that the treatment had no detectable effect on Bartonella muris. The toxicity of the sulfanilamide seemed to be a factor contributing to the mortality.

11354

Effect of Thymectomy at Birth on Spermatogenesis in the Albino Rat.*

JAMES C. PLAGGE (Introduced by Carl R Moore) From Hull Zoological Laboratory, The University of Chicago

Shay, et al, 'reported severe retardation of spermatogenesis following roentgen destruction of the thymus during the first few days of the rat's life. It would appear that if X-ray treatment of the

^{*}This investigation has been aided by a grant from the Dr Wallace (and Clara A. Abbott Memorial Fund of The University of Chicago

¹ Shay, Harry, Gershon Cohen, Jacob, Fels, Samuel S. Meranze, David R., and Meranze, Theodore, J. Am. Med. Assn., 1939, 112, 290

11353

Treatment of Bartonella muris Infections with Sulfanilamide.

FREDERICK E. EMERY.

From the Department of Physiology, School of Medicine, University of Buffalo, Buffalo, N. Y.

Splenectomy in rats is often followed by anorexia, anemia, hematuria, emaciation, and death. The cause of these severe symptoms is known to be a parasitic infestation commonly spoken of as *Bartonella muris* infection.^{3, 2, 3} Although the treatment of this condition is unsatisfactory, certain substances are beneficial, such as, arsenicals,⁴ copper and iron,⁵ and extracts of spleen.⁵

The beneficial effect of sulfanilamide and related compounds in distemper has been shown in dogs by Marcus and Necheles, and in ferrets by Dochez and Slanetz. Negative results have been noted in canine distemper and in rats infected with *Trichinella spiralis*. The wide use of sulfanilamide in these, and in other blood infections, led to the present experiments with *Bartonella muris*.

The rats used in these experiments were nearly full grown albino males. They were kept in a room having a constant temperature of 26°C with a variation of 2°C. Their diet consisted of Purina dog chow and water. Splenectomy, performed under ether anesthesia, was done through a shaved area of the skin, sterilized with alcohol. The same day, blood from a splenectomized rat suffering from an acute infection of Bartonella muris was injected intraäbdominally. Five cc of blood from the donor were mixed with 18 cc of 0.9% NaCl and 2 cc of a 25% solution of sodium citrate, making a total of 25 cc. Each recipient received 5 cc of this mixture. A 1% suspension of sulfanilamide was given intraäbdominally twice daily for 5 days in 5 cc amounts or a total daily injected dose of 100 mg. Also, about 20 mg daily were eaten with ground wheat. This made a total

¹ Lauda, E., Virchows Arch. path. Anat., 1925, 258, 529.

² Ford, W. W., and Eliot, C. P., J. Exp. Mcd., 1928, 48, 475.

³ Emery, F. E., et al., Endocrinology, 1940, 26, 167.

⁴ Mayer, M., ct al., Klin. Wschr., 1926, 5, 559.

⁵ Perla, D., and Marmorston-Gottesman, J., J. Exp. Med., 1932, 56, 777, 783.

⁶ Marcus, P. M., and Necheles, H., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 385.

⁷ Doehez, A. R., and Slanetz, C. A., Science, 1938, 87, 142.

⁸ Dickerson, V. C., and Whitney, L. F., Proc. Soc. Exp. Biol. And Med., 1938, 38, 263.

⁹ McCoy, O. R., Proc. Soc. Exp. Biol. and Med., 1938, 38, 461.

TABLE I. Spermatogenesis in Ruts Thymsectomized at Birth.

^{*250} seminiferous tubules examined in each animal,

thymus of newborn rats exerts such an influence on the testes, then complete surgical removal of the thymus at the same age would achieve similar results. This paper reports results of thymectomy in newborn albino rats with special reference to the progress of spermatogenesis.

Methods and Materials. The histological criterion for spermatogenesis used was that reported by Moore² in which the "sperm-head stage" appears between 33 to 35 days of age.

Forty-six male albino rats, representing 11 litters, were used. In all cases the litter was employed as the unit. The testes of thymectomized rats were compared with those of unoperated or sham-operated littermate controls. The thymus was removed before the animal was 24 hours old. Operations were performed under ether anesthesia or after chilling the animal. A mid-ventral section of the anterior end of the sternum was made back to the 4th intercostal space. Only those cases have been considered in which the thymus was removed in toto without surgical complications; in which no accessory cervical thymic tissue was seen at the time of the operation; and in which histological examination of suspected thymus rests, recovered at autopsy and sectioned serially, proved not to be thymus tissue. Both thymectomized animals and their littermate controls were weighed at 5-day intervals and were sacrificed at 33, 37, and 38 days.

Testes and seminal vesicles were weighed fresh and fixed in Bouin's solution. Complete cross sections of testes were cut at 7 micra and stained with Ehrlich's hematoxylin. Fifty seminiferous tubules were inspected in each of 5 different sections. The percentage of tubules containing sperm heads in the 250 tubules thus examined per animal was recorded as an index of the amount of spermatogenesis that had taken place.

Effect on testis. Table I presents data recorded for 46 rats. 25 of which were completely thymectomized at birth. The results of sperm head counts in exactly 250 seminiferous tubules per animal are expressed in percentage. It will be seen that all 9 thymectomized rats from 4 litters sacrificed at 33 days of age failed to show any sperm heads in the testes. In 5 of the 7 littermate controls, autopsied at the same time, sperm heads were completely missing. In 2 of the controls, 0.8% of the tubules contained sperm heads.

Out of 14 thymectomized rats sacrificed at 37 days of age, 12 showed sperm heads in 10% to 42% of the tubules examined, and 2 (Nos. 118 and 119) failed to exhibit sperm heads. Two unop-

² Moore, Carl, R., Am. J. Anat., 1936, 59, 63.

TABLE I. Spermatogenesis in Rats Thymeetomized at Birth.

²⁵⁰ seminiferous tubules examined in each animal.

erated littermate controls of the latter (Nos. 120 and 121) also revealed no sperm heads. One other control (No. 98) failed to show sperm heads on this day, whereas 9 had sperm heads in from 5% to 37% of the tubules observed.

In one litter of 4 males sacrificed at 38 days of age, both the 2 operated and the 2 control animals showed sperm heads in 24% to 33% of the tubules.

Neither absolute nor relative fresh testicular weights were influenced by thymectomy. This is an interesting point in view of the fact that gonadectomy of newborn male or female rats causes a definite hypertrophy of the thymus.³

These results indicate that thymectomy of the newborn male rat neither accelerates nor retards the process of spermatogenesis as judged by the first appearance of sperm heads in the testis.

Effect on seminal vesicle. Gross and histological examinations were made of the seminal vesicles of thymectomized rats and their respective littermate controls. The histology of the seminal vesicle has been described as a reliable indicator of male hormone secretion. At 33 days of age both thymectomized and control rats showed granular cytoplasm in the epithelium of the seminal vesicle. At 37 and 38 days distinct secretion granules were found in the vesicles of operated and control animals. Furthermore, the absolute and relative fresh weights of the seminal vesicles were practically the same for both groups.

Individual body weights, recorded at 5-day intervals, gave no indication that thymectomy in rats at birth, if unaccompanied by surgical complications, has any influence upon general body growth

up to 37 days of age.

Summary. Twenty-five male albino rats from 11 litters were thymectomized at birth. These were sacrificed at the ages of 33, 37, or 38 days. Testes and seminal vesicles were compared with 21 unoperated or sham-operated littermate controls. The results indicate that complete thymectomy of newborn rats neither hastens nor retards spermatogenesis, hormone output or growth of the testis.

³ Plagge, James C., unpublished.

⁴ Moore, Carl R., Hughes, Winifred, and Gallagher, T. F., Am. J. Anat., 1930, 45, 109.

11355

Failure of Ovarian Hormones to Cause Mating Reactions in Spayed Guinea Pigs with Hypothalamic Lesions.*

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It has been reported that female cats with small lesions in the hypothalamus in such a position as to interrupt the supraopticohypophysial tract were not observed to come into heat and were never bred in the laboratory. It has more recently been found that the production of small lesions in a comparable part of the hypothalamus of the female guinea pig is followed by a complete lack of the mating response and in some cases also by disturbances in the ovarian cycle.2 Although the disturbances in the sexual cycles may be attributable to a secondary disruption of hypophysial function, the majority of the animals showed regular sexual cycles which were normal so far as the physical changes in ovaries, uteri and vaginae were concerned, suggesting that their hypophyses were functioning normally. present investigation was undertaken in order to determine whether the lack of the mating response in guinea pigs following hypothalamic lesions of the type described is due to an hormonal insufficiency or to the destruction of neural elements indispensable to the estrous or mating reflex.

Marrian and Parkes³ have shown that vaginal estrus may be brought about by an amount of estrogen which is insufficient to induce uterine changes or copulatory behavior. On the other hand, Dempsey and Rioch⁴ were unable to induce behavioral estrus in a guinea pig following removal of the brain rostral to a plane extending between the anterior limits of the superior colliculus and the posterior edge of the mammillary bodies, although the reflex arc remained intact when the ventral limit of the section was in front of the mammillary bodies. On the basis of this evidence they have postulated a sexual center located in the ventral hypothalamus at the

^{*} Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

¹ Fisher, C., Magoun, H. W., and Ranson, S. W., Am. J. Obstet. and Gyncc., 1938, 36, 1.

² Dey, F. L., Fisher, C., Berry, C. M., and Ranson, S. W., Am. J. Physiol., 1940, 129, 39.

³ Marrian, G. F., and Parkes, A. S., J. Physiol., 1930, 69, 372.

⁴ Dempsey, E. W., and Rioch, D. M., J. Neurophysiol., 1939, 2, 9.

level of the mammillary bodies. Bard,⁵ however, has reported that estrous responses may be elicited in cats following massive lesions in the posterior hypothalamus which destroy all known descending paths from that part of the brain, and believes that the integration of the reflex is a mesencephalic function.

A series of 27 young, adult female guinea pigs, weighing between 400 and 600 g, were ovariectomized. Following a recovery period they were each brought into full behavioral estrus several times by the subcutaneous injection of 12.5-15.0 IU of estrogent on hours 0, 24, 48, and 60, followed by 0.2 IU of progesterone; on hour 72, after the method described by Collins, Boling, Dempsey and Young.6 After the constancy of the response to ovarian hormones had been established in each animal, lesions were placed in the hypothalamus at the level of the posterior border of the optic chiasma with the aid of a Horsley-Clarke instrument bearing a unipolar electrode. Three lesions were placed in each animal, one in the midline and one on each side of the midline at a distance of one millimeter, by passing a direct current of 3.0 ma for 30 seconds. In 22 animals the lesions were placed 1 mm above the ventral surface of the brain, and in 5 animals the lesions were placed 6 mm above the ventral surface. Five of the animals with the low lesions failed to survive the operation. Gross inspection of the brains from these animals indicates that the lesion occurs just posterior to the optic chiasma. The remaining 22 animals recovered completely, grew normally, and remained in excellent condition for the duration of the experimental period. Aside from the diabetes insipidus which developed in some animals, and a transitory period of depression which lasted for approximately 12 hours after the operation, there were no criteria by which the operated animals could be differentiated from normal anestrous female guinea pigs.

At least 2 attempts have been made to induce estrus in 17 of these animals with lesions near the ventral surface of the brain, using the dose of ovarian hormones which was sufficient to alter the behavior of the animals before the lesion. None of the animals so treated showed either proestrous or estrous behavior. It was impossible to elicit the estrous reflex by manual stimulation of the vulva or the lumbo-sacral region of the back, and none of these animals would

⁵ Bard, P., Res. Publ. Assn. Res. Nerv. Ment. Dis., 1940, 20, 551.

[†] Theelin, through the courtesy of Dr. Oliver Kamm, Parke, Davis and Co.

t Proluton, through the courtesy of Dr. Erwin Schwenk, Schering Corp.

⁶ Collins, V. J., Boling, J. L., Dempsey, E. W., and Young, W. C., Endocrinology, 1938, 23, 188.

accept the male. All gave good avoiding responses to such stimulation, after the manner of a normal anestrous female. In subsequent trials, 8 of the animals were injected with double the usual dose of hormones and failed to show estrus, while 4 of the animals were injected with quadruple the usual dose of hormones and also failed to come into heat.

That the failure of the ovarian hormones to induce estrus in these animals is not due to a non-specific effect of destruction in the central nervous system is shown by the experiments of Bard, Bard and Rioch, Brooks, Dempsey. and Davis. In addition, the 5 animals which have had lesions placed 6 mm instead of 1 mm above the ventral surface of the brain have been brought into heat with the same dose of hormone which induced estrus before the lesion was made.

These results differ from those of Dempsey and Rioch⁴ and Bard.⁵ Dempsey and Rioch's localization of the sexual center is based primarily upon the results of acute experiments on one guinea pig and one cat. In their chronic experiments failure to induce estrus following the removal of the anterior hypothalamic region is attributed to the debilitating effect of the operation on the animal. In their acute experiments successive transections were made in the same animal at various levels of the brain stem either with a blunt spatula or with a small sucker. In such experiments the accuracy of the localization of a "center" depends entirely upon the accuracy with which the location of the destruction to the central nervous system can be determined. We believe it may be significant that out of the 5 cats reported upon by Bard, the lesion in the one animal which failed to come into heat extended farther forward than in the other 4 animals. Although the main body of the lesion in the cats involved all known descending tracts from the hypothalamus, the possibility of the conduction of descending impulses by other paths has not yet been ruled out.

Summary. Following appropriately placed lesions at the level of the posterior border of the optic chiasma, ovariectomized guinea pigs failed to respond to previously effective dosages of estrogen and progesterone. The results reported here indicate that the failure of these animals to show estrous behavior is not due to a lack of ovarian hor-

⁷ Bard, P., Am. J. Physiol., 1936, 116, 4.

⁸ Bard, P., and Rioch, D. M., Bull. Johns Hopkins Hosp., 1937, 60, 73.

⁹ Brooks. C. M., Am. J. Physiol., 1937. 120, 544.

¹⁰ Dempsey, E. W., Am. J. Physiol., 1939, 126, 758.

¹¹ Davis, C. D., Am. J. Physiol., 1939, 127, 374.

mones. It is possible that the lack of response to the hormones is a result of the destruction of a portion of the central nervous system which is indispensable to the integration of a complex behavior pattern. If further control experiments prove this to be the case, then the possibility must be considered that the integrating mechanism involved is located in the midventral portion of the anterior hypothalamus instead of the region of the mammillary bodies or the mesencephalic tegmentum.

11356

Selective Localization of Evans Blue (T1824) in Subplacental Portions of Entoderm in the Rat.*

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The dye, Evans blue (T1824), a non-toxic, azo-compound, isomer of trypan-blue, has been observed following intravenous injection to localize selectively in and about malignant neoplasms in animals¹ and in man² but does not localize selectively in and about benign tumors in man or animals.² In and about the malignant neoplasms it accumulates in the macrophages and fibroblasts of the stroma. It does not penetrate into the neoplastic cells themselves, whether they be carcinoma or sarcoma.

During the course of experiments to observe its localization in tumor-bearing rats a pregnant animal (about mid-term) was inadvertently employed. At necropsy 24 hours after intravenous injection of 4 mg of the dye dissolved in 1 cc distilled water, it was noted that in addition to the sarcoma there was marked selective concentration of the dye in that portion of the visceral entoderm subjacent to the disc-shaped placenta. The remainder of the visceral entoderm did not appear grossly to have localized the dye (Fig. 1). The uterine musculature appeared tinged lightly blue as did the placenta; the embryo, and the amniotic fluid contained no dye grossly visible.

To confirm these observations 7 pregnant female white rats were

^{*} This work was carried out under a grant from the Cancer Research Institute of the Chicago Woman's Club, Chicago, Illinois.

¹ Duran-Reynals, F., Am. J. Cancer, 1939, 35, 98.

² Brunschwig, A., and Clarke, T. H., Am. J. Path., in press.

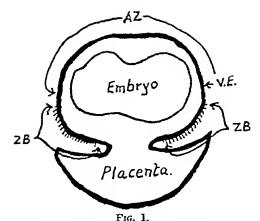


Diagram of cross-section of rat embryo in latter part of gestation. VE, visceral entoderm. ZB, subplacental portion of visceral entoderm with villous-like processes. These processes are not visible macroscopically. Selective localization of Evans blue was noted in this zone. AZ, anti-placental portion of visceral entoderm: cells are relatively lower in this region and no localization of the dye was noted here.

injected with similar quantities of dye, some at mid-term, and some just prior to the expected date of delivery. The animals were killed and at necropsy, 24 hours after injection, the concentration of the dye was observed in each instance as described above. Six pregnant females were injected with 4 mg of trypan blue. In 3 the localization occurred in the subplacental portion of entoderm as was found for Evans blue: in one the localization was not intense in this area, and in 2 it did not occur at all. Two pregnant females received intravenous injections of .5 cc India ink (diluted with water 1 to 3) and the latter localized definitely within the placenta as well as in the liver, spleen, bone marrow and some lymph nodes. The entire entoderm remained free from India ink.

Microscopic study of sections of the excised entoderms, portions of which had concentrated the Evans blue, and which were rapidly fixed, dehydrated and embedded to prevent loss of dye by diffusion, showed the dye within the columnar entoderm cells themselves as small aggregates of blue granules. The subplacental portions of the visceral entoderm are composed of tall columnar cells thrown into villus-like processes. The cells of the visceral entoderm became progressively flatter away from the placenta until over the area opposite the placenta where no dye was concentrated they are very low, flattened cells.

Discussion and Summary. The above described selective localization of Evans blue is of special interest since this localization in epithelial cells is in contrast to that observed when the dye concentrates selectively in and about carcinomas or sarcomas which localizations.

tion is in mesoblastic eells of the stroma—i. e., fibroblasts and macrophages. Such selective concentration might denote a special physiologic property of a portion of the visceral entoderm in the type of placentation represented in the rat.

Goldmann³ in his studies on intravital staining showed that trypan blue was eoneentrated in all of the entoderm in miee. However, storage of this dye was also observed by him in all of the reticuloendothelial system, of the mother, because of the relative enormous doses of the dye administered. Such very large doses precluded the demonstration of selective affinity for the dye on the part of certain tissues, as for example, the subplacental visceral entoderm. In his excellent monograph entitled "The Localization of Disease" Burrows4 illustrates in colors the exposed viseera of a pregnant rat injected with isamine blue. The uterine horns have not been opened and the dye appears localized in and about the placenta. The illustration was published to indicate specific concentration of the dve in the placenta. From our own studies in which the gross appearance of the unopened pregnant uterus was similar to that depicted by Burrows, we would raise the question of whether the dye he used was also concentrated not in the placenta primarily, but rather in the subjacent visceral entoderm as occurred with Evans blue in the observations reported above.

11357 P

Use of Orally Administered Desiceated Thyroid in Production of Traumatic Shock.

R. J. Schachter and J. Huntington. (Introduced by N. Kleitman.)

From the Department of Physiology, University of Chicago.

In an attempt to study the effect of adrenal cortical extracts on traumatic shock, we observed that normal anesthetized dogs. following repeated trauma to the limbs, testicles and gut, did not go into shock at least within 8 hours. We discussed this problem with Dr. Ivy, who had produced shock in dogs by trauma, and he suggested that a possible reason for his ability to induce shock in dogs might

³ Goldmann, E., Beitr. zur Klin. Chirurg., 1909, 64, 192.

⁴ Burrows, II., Localization of Disease, Wm. Wood & Co., London, 1932.

¹ Ivy, A. C., Am. J. Physiol., 1920, 51, 197.

have been due to the fact that most of the dogs he worked on had hyperplastic thyroid glands.

Following this information we performed the following experiments:

- 1. Thirty apparently normal dogs were anesthetized with ether, and blood pressure recorded by connecting the carotid artery with a mercury manometer. The limbs and testicles of these dogs were traumatized by 100 blows with a wooden mallet. We also manipulated the gut from ½ to 1 hour.
- 2. In another series of experiments 18 dogs were fed \pm g of desiccated thyroid tissue per kg of body weight per day for 1 week, then were anesthetized, and treated as in the first series, blood pressure recorded, and their intestines manipulated from 15 to 20 minutes. The average results of both series of experiments are recorded on the graph (Fig. 1).

The graph shows that normal dogs after severe trauma, did not go into shock, while experimental hyperthyroid dogs, with much less trauma died of shock within 3½ hours. The dogs which were fed thyroid were in apparently good condition. Although we did

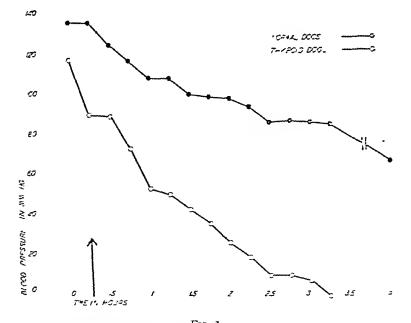


Fig. 1.

Upper line shows average blood pressure of 30 normal dogs. Low-r line shows average blood pressure of 18 hyperthyroid dogs. The arrow indicates the blood pressure 15 minutes after trauma.

not measure the B.M.R.'s of these dogs, studies on other dogs showed that this amount of thyroid usually increases the B.M.R. from 15 to 20%.

Unbeknown to us, Hepler and Simonds² had reported that dogs which were fed thyroid showed a greater drop in blood pressure than did normal dogs, when the hepatic veins were occluded for short intervals.

Further studies are being carried on to determine what changes occur that make it possible to produce traumatic shock in experimental hyperthyroid dogs.

Summary. The authors found that prolonged manipulation of the intestines of normal anesthetized dogs, does not produce shock. However, when dogs are fed .4 g of desiccated thyroid per kg of body weight per day for 1 week, these animals on manipulation of the intestines from 15 to 20 minutes, go into shock quite readily.

We wish to thank Dr. Carlson for his interest and advice during the course of these experiments.

11358 P

Accessory Growth Factor Requirements of Some Members of the Pasteurella Group.

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Most members of the Pasteurella group of bacteria develop satisfactorily in meat infusion-peptone media but fail to grow in simpler media made from hydrolyzed purified protein or in synthetic media. The substances in infusions of meat, other tissues or yeast which are needed for growth have not been previously identified. Accordingly we wish to report preliminary results of a study of the accessory growth factor requirements of some members of this group of organisms in which it will be shown that nicotinamide, pantothenic acid and, in some cases, the butyl factor for Clostridia are needed for prompt development.*

Seventeen typical Pasteurella strains were used. These were stock

² Hepler, Opal E, and Simonds, J. P., Arch. Path. 1938, 25, 149.

^{*} We are indebted to Dr. E. C. Snell and Dr. R. J. Williams for the samples of pantothenic acid used in these experiments and to Dr. W. H. Peterson for the butyl factor.

laboratory cultures which had been secured from different sources. They were isolated originally from hemorrhagic septicemias in various species of animals. The results presented in this report apply only to the typical strains of animal origin and not to other species at times included in this genus.

The basal medium consisted of a 0.5% solution of hydrolyzed purified gelatin to which was added a supplement of 8 amino acids, 0.3% glucose, 0.5% NaCl, 0.2% K2HPO4, 0.005% MgSO4 and 0.001% CaCl2. To this was added 1 cc of Hoagland salt mixture per liter of medium. The amino acid supplement consisted of 20 mg each of valine, tyrosine, tryptophane, cystine, methionine and histidine and 15 mg each of serine and threonine per liter. The medium was adjusted to pH 7.0 with N NaOH solution and tubed in 5 cc quantities.

The accessory growth factors were sterilized by filtration and added aseptically to the basal medium. In the first tests a mixture of known substances was used on the assumption that perhaps some of them might be required by these organisms. This mixture consisted of nicotinamide, diphosphopyridine nucleotide (cozymase), thiamine, thiamine diphosphate (cocarboxylase). riboflavin, beta-alanine, pantothenic acid, vitamin B₆ hydrochloride, nicotinamide methiodide, inositol, glutamine and sodium pyrophosphate.

The Pasteurella strains did not develop in the basal medium. Upon addition of the accessory mixture most of the cultures developed readily. Evidently one or more of the added factors was needed by these types. By simplifying the accessory mixture it was found that nicotinamide (or cozymase) and pantothenic acid were required for growth. Neither alone was effective. Two samples of pantothenic acid were used. Results obtained with one sample of 20% purity were duplicated with another of 70% purity. Pantothenic acid could not be replaced by beta-alanine. Tests with several characteristic strains showed that continuous cultivation through successive transplants was accomplished readily in the presence of nicotinamide and pantothenic acid.

Thirteen of the 17 cultures gave results essentially similar to those of *P. avicida* and *P. boviseptica* I shown in Table I. The other 4 cultures produced a scantier though still distinct growth, indicating that other factors or conditions were needed for ready cell multiplication. It was found that addition of the butyl factor for Clostridia¹ (probably biotin²) caused prompt and vigorous growth of

McDaniel, L. E., Woolley, D. W., and Peterson, W. H., J. Bact.. 1939, 37, 259;
 Woolley, D. W., McDaniel, L. E., and Peterson, W. H., J. Biol. Chem., 1939,
 131, 381.

² Snell, E. E., and Williams, R. J., J. Am. Chem. Soc., 1939, 61, 3594.

TABLE I. Effect of Growth Factors upon Development of Several Pastcurella Species of Animal Origin.

		Pasteurella -						
	Amt	avieida		boriseptica I		boviseptica 18		
Basal medium with addition of:	added, µg per cc af medimn	da;	ys* 2	da 1	ys 2	d	ays	
Nothing (control)								
Nicotinamide	0.1							
Pantothenic acid Nicotinamide plus	0.1					_		
pantothenic acid Nicotinamide plus	0.1 each	+++	+++	+++	+++	-1-	+	
beta-alanine	0.1 each							
Butyl factor	0.15						_	
Nicotinamide plus pantothenic acid	$0.1 \\ 0.1$							
plus butyl factor	0.15	+++	+++	+++	+++	++	+++	

^{- =} No visible growth, + = very light turbidity just at point of visibility,

3 of the remaining 4 cultures. The growth of P. boviseptica 18 (Table I) is an example of the effect of addition of butyl factor.

On substitution of a mixture of 18 amino acids for the hydrolyzed gelatin solution it was found that with but two exceptions all of the cultures could be grown in the presence of nicotinamide, pantothenic acid and the butyl factor. As far as we are aware cultivation of these organisms in an amino acid medium with the addition of known accessory substances has not hitherto been accomplished.

11359

Normal and Abnormal Prothrombin Levels.

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While low fibringen levels have been reported in pernicious anemia, scurvy, pellagra, acute yellow atrophy, and myeloid dyscrasias, there are not available adequate data as to the prothrombin levels of such diseases in man. Quick1 postulates that the blood in acute yellow atrophy of the liver may be as deficient in

⁺ to +++ = light to pronounced turbidity.

*All cultures were held for 10 days at 37° and observed at frequent intervals.
Usually there was no change after the second day.

¹ Quick, A. J., Am. J. Med. Sci., 1940, 199, 123.

prothrombin as in fibrinogen, and regards it highly improbable that a prothrombin deficiency can be produced in the adult by dietary means. It must be remembered that pellagra and sprue are both commonly associated with rather characteristic dietary habits, and evidence has already been offered for prothrombin deficiency in sprue.² and that for hypoprothrombinemia in the absence of jaundice in man due to inadequate vitamin K intake.³ Likewise, there exist bone marrow and intestinal relationships in sprue, pernicious anemia and pellagra.

The present report was undertaken on a small series of cases to determine the average normal limits of prothrombin clotting time in normal adult subjects and in treated and untreated patients suffering from unrelated diseases. The amount of prothrombin was estimated by the methods of Quick^{4, 5} and Warner, 6 and compared with the prothrombin index of whole blood. A total of 94 clinic and hospital patients, and 17 adult normals were observed over a period of 3 months. Six tests were performed on each concentration of 10% and 5% thromboplastin, with blood taken from the median basilic vein. The potency of the thromboplastin was estimated on the normal adults and was carefully prepared. The data are expressed in the number of seconds required for the clot formation to appear at 37°C with each lot of freshly prepared thromboplastin.

It has been shown by previous investigators that the normal prothrombin time is 16 to 19.⁴ 20.⁸ and 25⁷ sec. with an error of only 5%. Warner, et al:, with a plasma dilution method to evaluate the deficiency in prothrombin units have an approximate variation of less than 5%. It seems significant to compare the 3 tests for an index of normal with fresh blood and plasma under ideal conditions.

Table I illustrates the average values for prothrombin clotting time in normal and pathologic patients.

Patients with undiagnosed blood dyscrasias were omitted from the final series. Although the experimental error in the series by the wet and dry methods occasionally exceeded 5-10%, the results none-

² Clark. R. X., Dixon, C. F., Butt, H. R., and Snell, A. M., Proc. Staff Meet. Mayo Clin., 1939, 14, 107.

³ Kark, R., and Lozner, E. L., Lancet, 1939, 2, 1162.

⁴ Quick, A. J., Am. J. Med. Sci., 1935, 190, 501.

⁵ Quick, A. J., Am. J. Physiol., 1936, 114, 282.

⁶ Warner, E. D., Brinkhous, K. M., and Smith, H. P., Am. J. Physiol., 1936, 114, 667.

⁷ Aggeler, P. M., and Lucia, S. P., PROC. Soc. EXP. BIOL. AND MED., 1938, 38. 11.

⁸ Kato, K., and Poncher, H., J. A. M. A., 1940, 114, 9, 749.

TABLE I.

	1	See, with thromboplastin		Whole	% of	
No. of	·	10%	5%	blood	normal	
cases	Diagnosis	Wet	Dry	index	(Warner)	
S	Sprue	38	30	1.0	88	
9	Thrombocytopenic purpura	39	32	1+	80	
6	Polycythemia	29	30	1.0	86	
3	Microcytosis	28	25	1.0	92	
8 9 6 3 5	Acute pernicious anemia (untreated	1) 30	23	1.0	96	
12	Pernicious anemia with cord lesion	s 27	25	1.0	90	
16	Pernicious anemia without					
	cord lesions (treated)	27	30	1.0	82	
9	Alcoholic polyneuritis	33	30	1+	82	
$\frac{9}{1}$	Sprue (relapsing)	60	31	1+	76	
1	Parathyroidectomy (under					
-	treatment)	20	29	1.0	100	
4	Pellagra with alcoholism	29	26	1.0	94	
	Tabes with malaria	30	30	1.0	86	
ï	Partial gastreetomy (Ca)	28	32	1+	82	
3 1 5 3	CNS Lucs with malaria	32	28	1.0	89	
3	CNS Lues without malaria	30	32	1+	84	
	Sickle cell anemia	23	19	1.0	92	
ī	Lymphogranuloma inguinale	26	21	1.0	96	
3	Aplastic anemia	30	33	1+	78	
ő	Diabetes mellitus	25	27	1.0	90	
4 1 3 2 1 2 2	Splenectomy	34	30	1.0	86	
ĝ	Hodgkins Disease	32	29	1.0	86	
ő	Brucellosis	32	26	1.0	92	
17	Normal	26	28	1.0	94	

theless, under the ideal conditions show that the clotting time of prothrombin in the normal adult ranges up to 30 sec. as the upper limit with a prothrombin index of 1.0 for whole blood clotting. If 25-30 sec. is taken as the upper limit of normal, closer correlation may be found to exist with the other tests. The recent report of Kato and Poncher using the microprothrombin test of Kato⁸ on mature and immature infants, shows that the most mature infants likewise fall in the 25-30 sec. range. This seems to substantiate the results reported here.

At times it is difficult to account for the high readings given by dry thromboplastin, and low readings or vice versa from the wet solution prepared from the same source, with more uniform results occurring in this series with the dried material, if the only variable factor is the quality and amount of the prothrombin of the test plasma. Comparison of the prothrombin index of whole blood yields correlation to the studies reported here. Grossly abnormal tests if repeated will establish the general average range which has been observed to be 25 sec. plus or minus in the pathologic cases reported here and not above 30 sec.

None of the patients received synthetic vitamin K in therapy

and depended upon its content in their food. Nicotinic acid and vitamins B₁ and riboflavin were given to the pellagra patients. Alcohol-polyneuritis patients received vitamins B₁ and riboflavin and adequate food.

The sprue patients are interesting, since none of them received synthetic vitamin K and maintained adequate prothrombin levels. A case of sprue (relapsing) with marked diarrhea showed an almost normal prothrombin level. The sprue cases received liver extract

and yeast as did all pernicious anemias.

Despite the fact that prolonged readings have been observed in individual cases of pernicious anemias the general average reveals no marked deficiency of prothrombin. This apparently holds true for pellagra and polycythemia, treated and untreated. In a case of aplastic anemia following anti-luetic therapy, the prothrombin level was slightly reduced. While none of these patients enjoy maximum health, it is significant that an approximately normal or slight diminution of the prothrombin level is found even during characteristic exacerbations of their diseases. The whole blood clotting index may even be less than 1.0, illustrating that the volume per volume content of prothrombin in whole blood and plasma is not very significant.

Conclusions. (1) From the data presented the normal range of prothrombin-clotting time has been observed to be from 25 to 30 sec. Values above 30 sec have given a whole blood index of 1.0 plus. These results yield closer correlation to other methods for estimating the prothrombin level. (2) Treated and untreated patients with pernicious anemia, pellagra and other pathologic conditions have similar normal prothrombin values. Treated sprue patients gave similar results. In a case of sprue (relapsing) with persistent diarrhea, the prothrombin level was found to be almost normal. (3) When abnormal variations occur in wet or dry readings, it may be more accurate to depend upon the whole blood clotting index.

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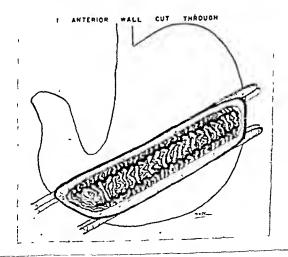
A New and Simple Method for Preparing Large Pavlov Pouches.*

F. Neuwelt, W. H. Olson and H. Necheles.

From the Department of Gastro-Intestinal Research, Michael Reese Hospital, Chicago.

The original method of Pavlov for the preparation of Pavlov pouches in dogs has been criticized recently by Hollander and Jere-They devised a new technic in which practically the entire vagal supply to the pouch was left intact. Their method yields excellent pouches, but is difficult and time-consuming. In our hands the eversion of the entire stomach through a small incision seemed to produce marked surgical shock, and increased greatly the danger of peritoneal contamination. Suturing of the mucosa was difficult and perforation between main stomach and poneh likely to occur. We therefore devised a simpler operation which yields big pouches with large amounts of secretion, which we feel worthwhile to make known to other workers in this field.

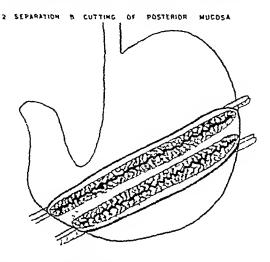
Procedure. Dogs were fasted for 24 hours and anesthetized with morphine-atropine and ether, or sodium pentoharbital. The stomach was exposed through a left reetus incision. The blood vessels at



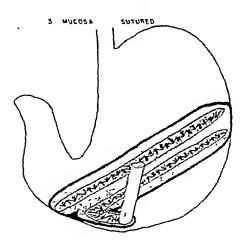
^{*} Aided by the A. B. Kuppenheimer Fund.

¹ Jeremin, E. E., and Hollander, F., Proc. Soc. Exp. Biol. And Med., 1938, 38, 139; Hollander, F., and Jeremin, C. E., PROC. Soc. EXP. BIOL. AND MED., 1938, 39, 87.

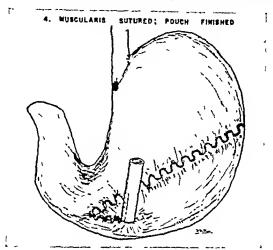
the greater curvature, below the angulus, were ligated and cut. Rubber-covered elastic clamps were put across the lower one-third of the fundus (Fig. 1). A straight incision through the entire anterior wall was performed (lately we cut only 4/5 of the anterior wall). The mucosa of the posterior wall was cut and about 1 cm was dissected away from the muscularis on each side of the incision (Fig. 2).



The main stomach was closed by an inverting running suture of the mucosa. The mucosa of the pouch was similarly closed, beginning at the upper end of the incision. The elastic clamp was removed, when about ½ of the pouch had been closed. At the lower pole of the



pouch a brass cannula (recently stainless steel) was inserted, and the submucosal suture carried in such a way that an angle was formed (Fig. 3). This is important because it avoids perforation between pouch and main stomach by the round base plate of the cannula. Muscularis of pouch and main stomach were united by Lembert suture (Fig. 4). Omentum was wrapped round the base of the



cannula. A small skin incision was made to the right of the midline at the height of the greater gastric curvature. A sharply pointed troear with sleeve was pushed through this opening, the trocar withdrawn, the cannula pushed into the sleeve and the latter withdrawn. Iodized gauze was placed round the external base of the cannula and a collar attached to it to prevent its slipping back into the pouch. The abdomen was closed in the usul way. Twenty-four hours after operation the iodized gauze was taken off, and the collar move up, in order to prevent pressure on the skin. The wounds were washed with hydrogen peroxide. One to 2 weeks after operation the dogs were ready for use. Active appetite secretion and immediate response to a meal characterized them as Pavlov pouches, i. e., pouches with good vagal supply. Intravitam and postmortem inspection of a number of these pouches showed no irritating effects of the metal cannula. Strong adhesions had formed between the neck of the pouch and the parietal peritoneum round the cannula.

Secretion is collected in 2 ounce glass bottles with serew cap. A collar is soldered on top of the cap, which is attached to the cannula by a serew. The weight of the bottle is supported by straps attached to the serew cap, which are fastened over the back of the animal.

TABLE I. Male Dog, 25 kg, 30-Min. Samples.

Y*-1	Acidity clinical units			
Volume ec	Free	Total		
0,2	0	7		
Fed 2	200 g Pard, 200 ce wa	ter		
35	140	154		
45	150	161		
48	149	161		
53	152	165		
61	157	169		
45	154	165		
42	158	169		
30	149	158		
23	162	172		
11	132	146		

The secretion is water clear. An example of secretion to a meal consisting of ½ pound of Pard dog food with 200 cc of water is given below.

Table I demonstrates that the pouch in the fasting dog has a minimal amount of secretion (0.07 cc per minute), no free and a minimal amount of total acid. Following a meal, free and total acid reach high values within the first half-hour and stay near the physiological maximum of acid secretion for 4½ hours. The total volume of fluid secreted during this period amounted to 382 cc. i. e., 1.4 cc per minute. Likewise the response of these pouches to histamine is excellent.

The posterior wall of the pouch has a complete vagal supply. The greater part (4/5) of the vagus branches of the anterior wall has been cut. In view of the excellent secretory performance of the pouch one can assume that the submucous plexus of the anterior wall receives sufficient vagal innervation from the remaining 1/5 of its connection with the musculature of the anterior wall, and from complete muscular bridge with the posterior wall of the main stomach. Dogs with such pouches have been in use in this laboratory for one year, and their pouch secretion has not changed. They are in fine physical condition and have no erosions or irritation round their cannula.

Summary. A new, simple and efficient method for preparation of Pavlov pouches in described, leaving intact more than 50% of the normal vagus supply to the pouch. The pouch has hardly any basal (fasting) secretion, with no free and very little combined acid. These pouches respond with maximal secretion, both volume and acidity, to food and histamine.

11361

Experimental Cinchophen Ulcer.*

FRANK NEUWELT AND H. NECHELES.

From the Department of Gastro-Intestinal Research, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

The cinchophen ulcer has been studied extensively since the first observations of Churchill and Van Wagoner. Stalker, Bollman and Mann have examined the problem of the cinchophen ulcer most thoroughly, and have reported their findings in several recent publications. Our laboratory has been interested in this problem for evaluation of ulcer therapy, and herein are presented our observations, some of which are at variance with previous findings.

Chronic Feeding Experiments. Seven dogs weighing from 8 to 14 kg were fed a diet of kitchen scraps (without bones), white bread, yeast, bone meal, salt, and cod liver oil to which was added

daily 1 to 5 g of cinchophen.†

Results. A 9.5 kg female white Spitz died after receiving 1 g of cinchophen daily for 12 days. Necropsy revealed a large perforated ulcer surrounded by smaller ulcerations on the proximal posterior aspect of the pyloric canal. A second white Spitz received 3 g of cinchophen daily for 10 days. The animal's weight declined from 8 to 6 kg, and it was found dead on the morning of the 11th day. Autopsy showed empyema of the chest and numerous small ulcerations of stomach and duodenum. One large shallow ulcer 3 cm in diameter was situated at the entrance of the pylorus. A third white Spitz (11 kg) received 25 g of cinchophen during a period of 20 days. The animal lost weight, refused to eat and became cachetic, but stools did not become tarry. Sixteen days after stopping the administration of cinchophen the animal died. At necropsy a number of acute small ulcers were found in the antral region of the stomach and in the first portion of the duodenum. A large,

^{*} Aided by the A. B. Kuppenheimer Fund.

¹ Churchill, T. P., and Van Wagoner, F. H., PROC. Soc. EXP. BIOL. AND MED., 1931, 28, 581.

² Stalker, L. K., Bollman, J. L., and Mann, F. C., Arch. Surg., 1937, 35, 290, 294.

³ Bollman, J. L., Stalker, L. K., and Mann, F. C., Arch. Int. Med., 1938, 61, 119.

⁴ Stalker, L. K., Bollman, J. L., and Mann, F. C., Arch. Surg., 1937, 34, 1172, 1174, 1176.

[†] We are obliged to Dr. D. A. Bryce of the Calco Chemical Company for supplying the einchophen.

deep chronic ulcer 2×2 cm in diameter was found near the pylorus on the posterior wall of the stomach.

The following 4 animals were of mixed breed. The fourth animal (9 kg) was fed a total of 14 g of cinchophen over a period of 14 days; no tarry stools, anorexia or loss of weight appeared. One month later 138 g of cinchophen were fed over a period of 48 days, at the end of which laparotomy with opening of stomach and duodenum failed to disclose peptic ulcerations. The stools were persistently negative for blood. The fifth dog (13.5 kg) was fed 4 g of cinchophen in 4 days. He then had complete anorexia but no tarry stools. We suspected a perforating, non-bleeding ulcer and laparotomy was performed. Stomach and duodenum were opened, but no ulceration, gastritis or duodenitis observed. A few days later the animal died of pneumonia which probably had begun earlier and had caused anorexia. Autopsy did not reveal gastrointestinal pathology. In dogs dying from pneumonia or distemper, gastritis and erosions are not infrequent. The sixth and seventh dog (12 and 14 kg) received 19 and 74 g of cinchophen in 19 and 24 days respectively. No tarry stools or other symptoms of ulcer were noted in either of these dogs.

It has been shown that the development of ulcer from oral administration of cinchophen is variable from the point of view of onset. Practically every experimenter in this field has found a small percentage of animals in which no ulcers developed under administration of cinchophen or certain other drugs even in high doses. In a comparable series of experiments by Stalker, Bollman and Mann ulcer developed in 11 out of 12 dogs that were not fed bone, in an average of 18 days after the administration of 36 g of cinchophen.2 In our series 3 dogs died and peptic ulcers were found at necropsy; the remaining 4 did not show evidence of ulceration during periods of administration of the drug of 4, 19, 24, and 47 days, although daily doses of approximately 3 and 4 g were given to the last 2 dogs. On the other hand the 3 dogs that died had been fed smaller doses of the drug over shorter periods of time (1, 2 and 3 g per day over 12, 20 and 10 days respectively). It is, therefore, of interest to point out that these 3 animals were white Spitzes. Perhaps this species of dog is more susceptible to cinchophen ulcers than others. A second point is that one of these animals died 16 days after the last dose of cinchophen and stomach and duodenum contained a number of acute ulcers, as well as one large chronic prepyloric ulceration. Stalker, Bollman and Mann² found that no new ulcers are formed and that chronic ones begin to heal practically immediately after eessation of administration of the drug. Hence, in the above dog, the etiologic cause for the acute and chronic ulcers remains open.

Effect of Cinchophen on Pouch Secretion. Four dogs with Heidenhain pouches, drained by a metal cannula were used. Their body weights were from 15 to 25 kg, with an average of 22 kg. Twice a week sceretion of the pouch was stimulated by subcutaneous injection of 1 mg of histamine acid phosphate every half-hour for a period of 3 hours. This method was chosen in order to establish the maximum secretory capacity of the oxyntic cells. Free and total acidity were titrated against dimethyl-amino-azobenzene phenolphthalein. Control studies of gastrie secretion were performed for several weeks, and cinehophen was administered intravenously as the neutral sodium salt, so as to be certain that the full dose was given and retained. The dose was 1 to 2 g per injection, given 4 to 6 times a week. Total amounts of drug administered to the 4 dogs were as follows: 32 g in 16 days, 32 g in 39 days, 12 g in 15 days, 50 g in 25 days, and one month later 28 g in 22 days. Several of the animals did not bleed from their pouch until 12 or more injections of einehophen had been given; at that time they showed symptoms of early gastritis, such as occasional tarry stools, vomiting and bleeding from the pouch, but analyses of histamine-stimulated secretion showed no increase in volume secretion at any time during the period of administration of the drug. As a matter of fact the amount of secretion decreased somewhat and became irregular. Free and total acidity also tended to diminish and fluctuate erratically, independent of occasional bleeding from the pouch. one dog the highest values for free and total acidity were observed when bleeding began, just one day before death. Autopsy revealed peritonitis from perforated duodenal ulcer, just beyond the pylorie ring. Gastritis was present in main stomach and pouch; this was the only animal in the series which died or perforated.

Stalker, Bollman and Mann⁴ found an increased volume secretion in their pouch dogs following administration of einchophen when symptoms of early gastritis, such as tarry stools and occasional vomiting appeared. When administration of einchophen was discontinued, a gradual decrease in the amount of gastric secretion to the normal level occurred within 2 weeks. They employed food as secretagogue for their pouch dogs and histamine for their dogs with intact stomachs. Gastric juice was collected continuously from their pouch dogs, but secretion tests on their dogs with intact stomachs was done every second or third day. The dogs of the latter group

(17 kg average weight) received one dose of 1.5 mg of histamine subcutaneously. Yet the results obtained in both groups of animals were identical with regard to increased volume of secretion. The administration of 0.5 g or more of cinchophen caused evidence of gastritis in every dog within 8 hours after the initial dose; when the dose was 1 g or more daily, ulcers developed in from 2-5 days and usually perforated.⁴

The discrepancy between our results and those of Stalker, Bollman and Mann can hardly be found in the fact that our technical procedures were different. Our pouch dogs (22 kg average weight) received 1 mg of histamine every half hour for 3 hours (total of 6 mg). We feel that an augmented volume of secretion would certainly have been found by this method. Our pouch dogs received roughly similar individual doses of cinchophen by vein and for longer periods than did the pouch dogs of Stalker, Bollman and Mann⁴ by mouth, i. c., larger total amounts of the drug, and yet only one animal died from perforated gastric ulcer while the rest survived. It has been shown that the drug acts after absorption, and not locally.5 or after excretion into the stomach from the blood.2 Therefore one cannot assume that oral or intravenous administration of cinchophen should have different effects. Furthermore, vomiting may cause loss of orally administered drug. It may be that our preparation of cinchophen varies in some way from that of the above and other workers.I

Summary and Conclusions. 1. White Spitz dogs seem more prone to develop cinchophen ulcers than other species of dogs. 2. Heidenhain pouch dogs receiving cinchophen intravenously show no increase in volume secretion under histamine stimulation.

⁵ Churchill, T. P., and Manshardt, D. O., Proc. Soc. Exp. Biol., and Med., 1933, 30, 825.

Cinchophen Calco, Lot No. 24331 and 29056 were used.

11362

Vago-Neurohypophysial Pressor Reflex.

D. G. Sattler. (Introduced by W. R. Ingram.)
From the Department of Anatomy, State University of Iowa.

It has been shown repeatedly that interruption of the supraopticohypophysial tract causes atrophy of the neurohypophysis with certain cellular alterations. If sufficient neurohypophysis is deprived of its nerve supply in this way, diabetes insipidus ensues (Fisher, Ingram and Ranson^t). These atrophic neurohypophyses have been found to be lacking in the hormonal substances produced by the normal gland, and it is generally held that diabetes insipidus is due to lack of an antidiuretic hormone. The question remains, is the innervation of the neurohypophysis a trophic one, or may it participate in the regulation of the functions of the latter? There is some evidence for neurogenic control of the neurohypophysis.

Theobald and Verney,² and Pickford³ have advanced indirect evidence to show that humoral and neural influences may cause the neurohypophysis to increase its output of antidiurctic substance. Haterius⁴ has confirmed this and added more direct evidence for participation by the pituitary. Gilman and Goodman³ found antidiurctic substance in the urine of dehydrated normal rats, as did Boylston and Ivy,⁶ the latter pointing out the similarity between the action of this antidiurctic substance and that of pitressin. Gilman and Goodman could not obtain similar results with dehydrated hypophysectomized rats. Ingram, Ladd and Benbow⁵ have offered evidence that while appreciable amounts of antidiurctic substance are exercted by normal cats in a state of dehydration, dehydrated cats with diabetes insipidus do not exercte such material. Walker,⁵ however, did not obtain such results. Gersh⁶ reports that "paren-

¹ Fisher, C., Ingram, W. R., and Ranson, S. W., Diabetes Insipidus, Edwards Brothers, Inc., 1938.

² Theobald, G. W., and Verney, E. B., J. Physiol., 1935, 83, 341.

³ Pickford, M., J. Physiol., 1939, 95, 226.

⁴ Haterius, H. O., Am. J. Physiol., 1940, 128, 506.

⁵ Gilman, A., and Goodman, L. S., J. Physiol., 1937, 90, 113.

⁶ Boylston, C. A., and Ivy A. C., Proc. Soc. Exp. Biol. and Med., 1938, 38, 644.

⁷ Ingram, W. R., Ladd, L., and Benbow, J. T., Am. J. Physiol, 1939, 127, 544.

⁸ Walker, A. M., Am. J. Physiol., 1939, 127, 519.

⁹ Gersh, I., Am. J. Anat., 1939, 64, 407.

chymatous glandular cells" of the neurohypophysis of rats increase in size and number in dehydration and at parturition.

Chang, Lim, *et al.*, 10, 11 have reported a series of interesting ob-

Chang, Lim, ct al., 10, 11 have reported a series of interesting observations made upon experimental animals in which the only connection between head and body was vascular. Stimulation of the central end of the cut vagus in such dogs caused characteristic elevations of blood pressure. Since acute hypophysectomy abolished this response, they ascribed this reflex to a liberation of a pressor principle from the neurohypophysis, indicating that the integrity of the reflex arc depended upon the presence of an intact supraopticohypophysial tract and anatomically obscure central intermediary neurons. In similar experiments increased production of oxytocic, glucogenic and antidiuretic substances was also described. These results are of such significance as to warrant confirmation. This communication deals with an attempt to confirm certain findings of these workers, and with further data from experiments carried out on dogs with chronically denervated neurohypophyses. The latter experiments were designed to rule out errors which may conceivably be introduced by the trauma and shock contingent upon acute hypophysectomy.

Methods. In all experiments nembutal anesthesia was given intravenously. Blood pressure was recorded from the femoral artery. After isolation of the carotids, jugulars, and vagi, and cannulation of the trachea, all other neck structures were crushed in a specially constructed vise according to the methods of Chang, et al. The artificial respiration rate was 8 respirations per minute. The blood pressure fall consequent to cord crushing was combated by the intravenous administration of Ringer's solution and 5 mg doses of ephedrine. By these means it was found possible to maintain the blood pressure at or above 70 mm Hg; experiments in which the basal pressure was lower than this level were discarded. Destruction of the spinal cord was checked at autopsy.

Results. 1. Acute experiments. After the technic of preparing such an animal was sufficiently developed, it was found that the rise in blood pressure on stimulation of the central end of the severed vagus was quite easily obtainable. In these experiments, acute hypophysectomy was carried out through an opening in the roof of the mouth so as not to disturb other intracranial structures. The

¹⁰ Chang, H. C., Chia. K. F., Hsu, C. H., and Lim, R. K. S., Chin. J. Physiol., 1937, 12, 309.

¹¹ Chang, H. C., Lim, R. K. S., Lu, Y. M., Wang, C. C., and Wang, K. J., Chin. J. Physiol., 1938, 13, 269.

11362

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⁵ Gilman, A., and Goodman, L. S., J. Physiol., 1937. 90, 113.

⁶ Boylston, C. A., and Ivy. A. C., PROC. Soc. Exp. Biol. And Mep., 1938, 38, 644.

⁷ Ingram, W. R., Ladd, L., and Benbow, J. T., Am. J. Physiol., 1939, 127, 544.

⁸ Walker, A. M., Am. J. Physiol., 1939, 127, 519.

⁹ Gersh, I., Am. J. Anat., 1939, 64, 407.

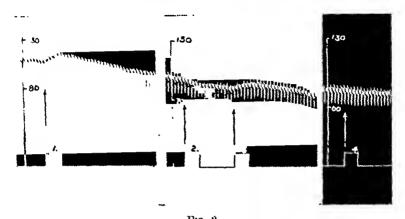


Fig. 2.

Blood pressure tracings from dog 14, with diabetes insipidus and the cervical spinal cord crushed. Responses to stimulation of the central vagus stump before and after cervical sympathetic ganglionectomy.

2. Experiments with dogs with chronically denervated neuro-hypophyses. In these animals the supraopticohypophysial tract was sectioned in the median eminence, using a subtemporal approach. Criteria for the completeness of the section were the occurrence of marked permanent diabetes insipidus and examination of microscopic sections of the infundibular region. These dogs were used to show further that the pressor response is abolished in the absence of neuro-hypophysial innervation, and to rule out any possible error caused by trauma and shock in the acutely hypophysectomized animals.

The following experiment is typical:

Dog No. 14. Before stalk section average urine output was 200 cc (Sp. Gr. 1.032). After section of the stalk average urine output was 2978 ce (Sp. Gr. 1.002). Following the 6th day there was a 4-day normal interphase after which the water intake and urine output rose to the previous high level. On the 16th postoperative day the spinal cord was crushed and stimulations were carried out (Fig. 2). At 1, faradic stimulation of the right vago-sympathetic trunk (coil at 5 cm) for 45 seconds. Immediate onset of a pressor effect. Between 1 and 2 the right superior cervical sympathetic ganglion was removed. At 2, stimulation of the right vagus for 45 seconds caused no change in blood pressure. At 3, stimulation of the left vago-sympathetic trunk for 45 seconds. Immediate pressor effect. Between 3 and 4 the left superior cervical sympathetic ganglion was removed. At 4, stimulation of the left vagus for 45 seconds caused no change in the blood pressure.

Similar results were obtained in 3 experiments. In addition a dog

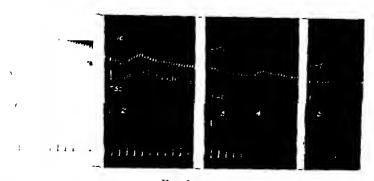


Fig. 1.

Blood pressure tracings from a dog with the cervical spinal cord crushed. Responses to stimulation of the central vagus stump, with the effect of cervical sympathetic ganglionectomy and hypophysectomy.

completeness of posterior lobe resection was confirmed by careful autopsy examination.

The results of a typical experiment are illustrated in Fig. 1. At 1, faradic stimulation (coil at 5 cm) of the left vago-sympathetic trunk for 45 sec. Note the sudden pressor effect upon which a second mild pressor phase is superimposed. Between 1 and 2 the left superior cervical sympathetic ganglion was removed. At 2, stimulation of the central end of the left vagus for 45 seconds. After a 30-second delay a rise in pressure occurs. Between 2 and 3 the hypophysis was removed through a previously made opening in the roof of the mouth. At 3, stimulation of the central end of the left vagus for 45 seconds. No response. At 4, the intact right vago-sympathetic trunk was stimulated for 45 seconds. Sudden onset of pressor response. Between 4 and 5 the right superior cervical sympathetic ganglion was removed. At 5, stimulation of the right vagus caused no response in blood pressure. Such results were obtained in 7 experiments.

Conclusions. In a normal dog, with the spinal cord crushed, stimulation of the central end of the vago-sympathetic trunk causes a pressor effect in the body. This effect seems to be of two components; a sudden rise (sympathetic effect) and a more delayed rise (vagus-neurohypophysis effect). That the elevated pressure resulting from stimulation of the vagus alone is effected through the pituitary is indicated by abolition of the response through hypophysectomy.

This experiment was tried on several cats with similar results but with great variability under the conditions. Technical obstacles

made the experiment difficult.

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A Modified Method for the Preparation of Renin.

W. D. Collings,* J. W. Remington, H. W. Hays and V. A. Drill (Introduced by W. W. Swingle.)

From the Biological Laboratory, Princeton University, Princeton, N. J.

Partially purified renin has been prepared in several laboratories from NaCl or cold acetone extracts of the kidney cortex.²⁻⁴ Recently modifications in our original extraction procedure² have been introduced which eliminate many of the more cumbersome manipulations involved, with considerable saving of time, without sacrificing the relative high potency of the final product.

Extraction Procedure. Demedullated kidneys are ground, frozen, and, before thawing, reground into 2% NaCl solution (10 liters to 3 kg tissue). It is possible to use fresh, unfrozen kidneys, but the extraction appears to be less complete. After the salt extract has been stored for 24 hours under toluene, the meat sludge is removed by straining and centrifuging (Sharples). The pH is then lowered to 4.5. After an interval of 12 to 24 hours to insure complete precipitation, the heavy precipitate is removed by centrifuging (Sharples) and filtering through Hyflo Super-Cel.† For easier handling in subsequent procedures, the filtrate, adjusted to pH 6.8. is concentrated in vacuo (maximum temperature 45°C) to a volume of 1 liter. The concentrate is filtered, 100 g NaCl are added and the pH is lowered to 2.0. The heavy precipitate is removed on a filter cake of Hyflo which is then suspended in 2 liters of water. After the pH is raised to neutrality and the suspension thoroughly mixed by a motor stirrer for about 30 minutes. Hyflo and any insoluble precipitate are filtered off and discarded. The filtrate is saturated with solid NaCl and the pH again lowered to 2.0. The precipitate is removed on a filter cake and redissolved in 500 cc N/10 acetate buffer at pH 5. As before, insoluble residue is discarded. At this stage two lots of extract (each representing 3 kg kidney cortex) are combined in the same 500 cc of buffer solution. Precipitation with

^{*} E. R. Squibb and Sons Fellow in the Biological Sciences.

¹ Helmer, O. M., and Page, I. H., J. Biol. Chem., 1939, 127, 757.

² Swingle, W. W., Taylor, A. R., Collings, W. D., and Hays, H. W., Am. J. Physiol., 1939, 127, 768.

³ Hessel, G., Klin. Woch., 1938, 17, 843.

⁴ Hill, J. R., and Pickering, G. W., Clin. Sci., 1939, 4, 207.

t Celite Filter Aid, Johns Manville Company, New York.

with diabetes insipidus in which cervical sympathetic ganglionectomy was not done yielded only typical sympathetic responses without evidence of vagus pressor responses. In one operated dog with a good transient but not a permanent polyuria, vagoneurohypophysial responses were not obtained; microscopic sections showed extensive but not quite complete degeneration of the supraopticohypophysial system.

Conclusions. Stimulation of the central end of the vago-sympathetic trunk in a dog with diabetes insipidus gives a pressor response. This pressor response is due to stimulation of the sympathetics of the head, since the response is abolished by sympathetic ganglionectomy. In the absence of the supraopticohypophysial connection the vagus-postpituitary reflex is not obtainable even under the best conditions, as when the basal blood pressure level is high.

3. In a number of acute experiments the infundibulum was stimulated directly with weak faradic current. Fine bipolar electrodes were used and introduced manually through a buccal opening. The spinal cords were completely crushed in each of these dogs. Striking elevations in blood pressure were obtained. These experiments supplement those of Clark and Wang¹² in which hypothalamic stimulation produced pressor effects in spinal cats, and offer further indication that the results of these workers were due to activation of the neurohypophysis.

Summary. 1. In dogs with only vascular connections between head and body, stimulation of the central end of the severed vagus causes blood pressure elevations in the body. Acute hypophysectomy abolishes this reflex. 2. This reflex cannot be obtained in preparations with chronic diabetes insipidus caused by interruption of the supraopticohypophysial tract. This rules out possible error due to shock, tranma, etc., consequent to acute hypophysectomy. 3. These results add to the evidence found elsewhere that the neurohypophysis is subject to nervous control mediated by the supraopticohypophysial tract.

¹² Clark, G., and Wang, S. C., Am. J. Physiol., 1939, 127, 597.

TABLE I.							
Some	Color	Reactions	οf	Renin.			

Reaction	Reactive group	Result
Biuret	peptide linkage	+
Millon	tyrosino	+
Xanthoproteic	benzene nucleus	+
Hopkins-Cole	tryptophane	+
Sullivan 's	cysteine, cystine	-
Ehrlieh-diazo	histidiné, tyrosine	+
Sakaguchi	arginine (guanidine)	+
Molisch	carbohydrates	<u> </u>
Benzidine	pentoses	

It will form a picrate and is destroyed rapidly by boiling and by protein denaturants. It is free from carbohydrate and responds to routine color tests as shown in Table I. The test for -SH groupings was negative in both native and denatured (boiled) protein and was absent also after treatment with NaCN. However, renin shows the presence of sulfur after sodium fusion.

Yield. The yield in total solids per kg kidney cortex is fairly constant at 150-180 mg. The variability in activity, however, is large, for reasons at present not known. The renin unit² has been defined as the amount of material, per kg body weight, which will produce a 40 mm rise in the mean blood pressure of the anesthetized dog. In the assay standard, 1 unit was the equivalent of 0.1 mg renin. In 39 successive lots of extract, the rise given by 0.1 mg renin, per kg body weight, has been between the extremes of 13 and 62 mm, with the mean at 34 mm. In other words, the average yield was 1450 units per kg fresh cortex. In our most potent extract (Table II) 1 unit represents 9.6 μg nitrogen.

TABLE II.
Renin Assay at Dosage of 0.1 mg per kg Body Weight.

Dog No.	1	. 2					7						Avg
Body wt, kg Initial B.P.,*	9.	7 9.	5 13.	1 12.	5 7.5	9.	5 7.	6 10.	7 12.5	11.6	12.6	9.4	10.5
mm Hg Peak B.P.,	108	114	115	103	88	89	122	93	146	95	125	96	108
mm Hg' Rise in B.P.,	175	165	177	209	174	139	178	139	199	156	172	157	170
mm Hg	67	51	62	106	86	50	56	46	53	61	47	61	62.2

^{*}B.P. readings are mean pressures obtained by intra-arterial needle puncture.

solid ammonium sulfate at 0.4 saturation is made 5 successive times, with the volume of buffer solution reduced at each step, c. g., 500, 400, 300, 200, and 100 cc. The first 4 precipitates are removed on filter cakes, the 5th is removed by centrifuging. The discarded supernatant should be color-free. When the final precipitate is not easily soluble in 40-70 cc water, to give a clear, light amber solution, ammonium sulfate precipitations are repeated from a volume of 50-100 cc. It is important that the volume of buffer solution be kept small, since inactive less soluble globulins are left behind at each filtration. The extract is now dialyzed at 6°C against distilled water. We have found that any precipitate forming in the dialyzer can be discarded as inactive. The final volume of 60-100 cc should have 10-15 mg solids per cc. Where the total solids are higher, further ammonium sulfate precipitations from the dialyzed solutions are usually made. The recovery is not quantitative, but inactive solids are often climinated by this step.

All pH adjustments are made by 10% HCl or 10% NaOH and are measured with a glass electrode. Stock reagents are kept in the refrigerator, and care is taken to keep the extract chilled as much as possible throughout its preparation.

Dialysis. A circulating dialyzer in the refrigerator is usually employed. However, no difference in the activity of the final product has been observed when the slower, standing dialysis (cellophane bag in a cylinder of water) is substituted. There is apparently no loss of potency, in the cold, even though dialysis is prolonged 3 to 4 days.

Stability. The extract is stored either frozen or in the lyophile state. The latter material is prepared by the Cryochem process⁶ and stored in the refrigerator. It has shown full activity when tested as much as 10 months after preparation. Frozen extract, especially if melted and refrozen repeatedly, shows a gradual loss of activity.

Preparation of Sterile Renin. The extract can be sterilized by filtering through a Jena glass filter and then rendered lyophile in sterile vials. A Seitz filter adsorbs all the active protein from the acid solution.

Chemical Properties. Renin, as prepared by the method described above, is a pseudo-globulin and is precipitated by 0.38 to 0.41 saturated ammonium sulfate at pH 5, by saturated NaCl, by 0.7 to 1.0 saturated magnesium sulfate and by the various protein precipitants.

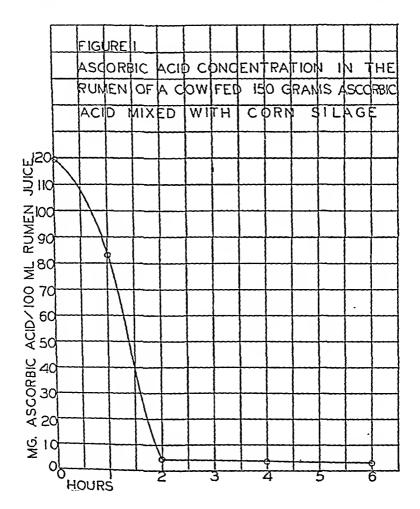
⁵ Taylor, A. R., Parpart, A. K., and Ballentine, R., Ind. and Eng. Chem., 1939, 11, 659.

⁶ Flosdorf, E. W., and Mudd. S., J. Immunol., 1938, 34, 469.

the cow was on a standard ration unsupplemented with the vitamin. A slight increase was noticed in the amount of ascorbic acid found in the 24-hour sample of urine for the periods during which the vitamin was administered.

A rapid and pronounced destruction of ascorbic acid in the rumen was demonstrated by removal and analysis of samples of the rumen contents at regular intervals after the cow had been fed (Figs. 1 and 2). Ascorbic acid added to rumen contents in vitro and stored in a dark-glass, stoppered bottle at 39°-42°C disappeared at much the same rate as that of the *in vivo* experiments.

These results are not in accord with the conclusions of Riddell and



11364

Destruction of Ascorbic Acid in the Rumen of the Dairy Cow.*

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Dutcher and coworkers,1 Hart, Steenbock and Ellis,2 and Hess, Unger and Supplee³ presented evidence to show that the diet of a dairy cow influenced the antiscorbutic potency of the milk produced. Using guinea pigs to test the antiscorbutic potency of the ration which was fed and to assay the milk which was produced, each of these groups of workers found that milk obtained from cows on a vitamin-rich ration was definitely superior in antiscorbutic value to the milk derived from cows on a vitamin-poor diet. These findings, though widely accepted, were disputed by Hughes and coworkers,4 who concluded from a series of experiments that the ration received by cows had no influence on the antiscorbutic property of their milk.

Since the development of chemical methods for the quantitative determination of the antiscorbutic factor, which was shown to be ascorbic acid, differences of opinion have arisen concerning the factors which have the greatest influence on the amount of vitamin C in milk. It is now generally agreed, however, that the vitamin C content of milk is independent of the season of the year and the ration of the cow. This fact has led to the present investigation of the fate of ingested ascorbic acid in the cow.

A rumen fistula was made in a Holstein cow. Experiments were performed in which this cow was fed (A) 100 g (2,000,000 International Units) and (B) 150 g of synthetic ascorbic acid mixed with corn silage; 100 g of ascorbic acid were also placed directly in the rumen through the fistula opening.

Similar results were obtained in all of the experiments. No increase was observed in the ascorbic acid values of the blood plasma and of the milk when compared with those values obtained while

^{*} Authorized for publication on March 19, 1940, as paper No. 963 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

¹ Dutcher, R. A., Eckles, C. H., Dahle, C. D., Mend, S. W., and Schaffer, O. G., J. Biol. Chem., 1920, 45, 119.

² Hart, E. B., Steenbock, H., and Ellis, N. R., J. Biol. Chem., 1920, 42, 383.

³ Hess, A. F., Unger, L. J., and Supplee, G. C., J. Biol. Chem., 1920, 45, 229.

⁴ Hughes, J. S., Fitch, J. B., and Cave, H. W., J. Biol. Chem., 1921, 46, L.

⁵ Kon, S. K., The Journal of Dairy Research, 1938, 9, 242.

in detecting dehydroascorbic acid as well as the reduced form of the vitamin.

The authors wish to acknowledge the assistance of Dr. J. F. Shigley, who performed the fistula operation, and to express their appreciation for the generous supply of ascorbic acid furnished by Chas. Pfizer and Company, New York.

11365 P

Effect of Electrotonus on Accommodation in Nerve.

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University.

Hill has proposed an approach to the response of excitable tissues involving two processes, one a rise of the "local potential" and the other a change of threshold called "accommodation", the rates of which are represented by the time constants "k" and " λ " respectively. Blair² has pointed out some theoretical inadequacies arising from investigations of the effects of electrotonus on rheobase and chronaxie (theoretically .693k), but in the absence of similar studies on λ , the extent of such limitations is not clear. Consequently, the present investigation of λ was undertaken.

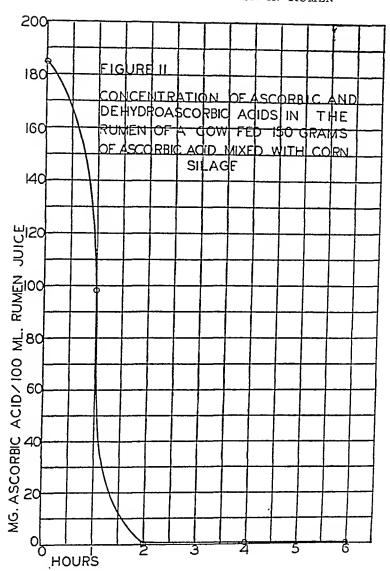
The technic described by Solandt³ employing exponentially rising currents was used to determine the λ of the sciatic nerves of Rana pipiens. The same nonpolarizable electrodes, 2 cm apart, were employed to produce a 2-second electrotonus and to apply the exponential currents. Special precautions were taken to minimize residual and progressive effects. Most experiments were performed at 20°C.

The chief results obtained are summarized in the accompanying figure. The ordinate represents the relative change in λ (i.e., the ratio of λ during electrotonus, λ_e , to λ of the normal nerve, λ_n) and in rheobase (i.e., the rheobase during electrotonus, V_e , divided by its normal value, V_n), while the abscissa is the intensity of electrotonus (E/ V_n) in rheobases. It can be seen from the continuous

¹ Hill, A. V., Proc. Roy. Soc. London, Ser. B, 1936, 119, 305.

² Blair, H. A., Cold Spring Harbor Symposia of Quantitative Biology, 1936, 4, 63.

³ Solandt, D. Y., Proc. Roy. Soc. London, Ser. B, 1936, 119, 355.



Whitnah⁶ who suggested that the rapid disappearance of vitamin C from the rumen of a cow fed large amounts of green rye was due to a quick absorption of the vitamin.

In making the above analyses, both the indophenol titration and the Roe furfural method were employed.⁷ The latter method was useful

⁶ Riddell, W. H., and Whitnah, C. H., J. Dairy Science, 1938, 21, 121.

⁷ Roe, J. H., and Hall, J. M., J. Biol. Chem., 1939, 128, 329.

curvature was noticed by Solandt, who was unable to account for this divergence from theory.

Comparison of the electrotonic effects which have been described with those obtained by Nivet* for chronaxie and rheobase indicates a possible relationship between λ and k which is contrary to the suggestion of their independence made by Hill and Solandt but which is not the simple direct one insisted upon by the Lapicques.⁵

Confirmation of the effect of electrotonus on λ is seen (1) in the observation by Parracke that accommodation at the anode is smaller than at the cathode, (2) in the decline of excitability following the initial rise during the passage of a linearly increasing current (Fabre') instead of a rise in excitability to a maximum which should theoretically be maintained, and (3) in the decrease of "Einschleichzeit" (which Hill has shown is related to λ) obtained by Schriever's with catelectrotonus.

11366

Occurrence of Tremors and Incoordination in Vitamin E-Deficient Adult Rats.

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Paralysis in adult rats grown and maintained on a vitamin E-deficient diet was first described in detail by Ringsted¹ and later by Burr, Brown and Moseley.² Einarson and Ringsted³ reported degenerative changes in the central nervous system and voluntary muscles, that were prevented but not cured by wheat germ oil. The

⁴ Nivet, M., C. R. Soc. Biol., 1934, 116, 1013; Ibid., 1939, 131, 262.

⁵ Lapicque, L., and M., C. R. Soc. Biol., 1937, 125, 260; Ibid., 1938, 129, 724.

⁶ Parrack, H. O., Am. J. Physiol., 1939, 126, 597; Proc. Am. Physiol. Soc., 52nd Annual Meeting, 1940, p. 142.

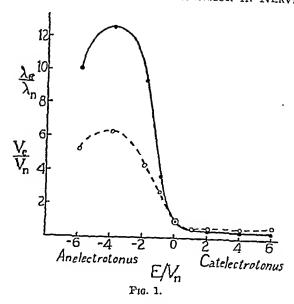
⁷ Fabre, P., C. E. Soc. Biol., 1934, 116. 1065.

⁶ Schriever, H., Zeitechr. f. Biol., 1932, 98, 123.

¹ Ringsted, A., Biochem. J., 1935, 29, 788.

² Burr, G. O., Brown, W. R., and Moseley, R. L., PEOC. Soc. Exp. Biol. AND Med., 1937, 36, 780.

³ Einarson, L., and Ringsted, A., 1938, Effect of Chronic Vitamin E Deficiency on the Nervous System and the Skeletal Musculature in Adult Rats, Oxford University Press.



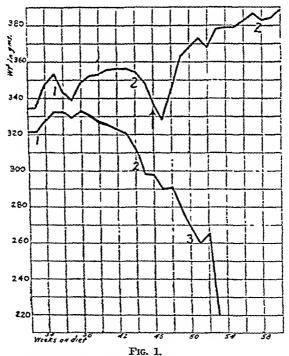
curve, which represents the modifications in λ , that λ is increased by an electrotonus and decreased by catelectrotonus. In none of the 168 measurements of λ during electrotonus was an exception to this found. It is obvious, too, that λ is a continuous function of the electrotonic intensity, a function which is similar to that of rheobase represented by the broken curve.

Such similarity is exactly contrary to the behavior to be expected if alterations of k and A alone govern the behavior of the nerve. For example, an increase in rheobase is often interpreted as being caused by faster accommodation. Experiments not involving electrotonus have also shown rheobase and λ to behave in such a theoretically unexplainable manner. Thus, unsoaked nerves of summer frogs mounted in a moist chamber at 29°C also exhibited an increase of both rheobase and λ with time; measurements before and after soaking in Ringer's solution often indicated the same. Furthermore, preliminary experiments indicate that although nerves soaked in calcium-rich Ringer's solution show an increase in rheobase, as expected from the concomitant decrease in λ , the order of magnitude of this increase is much larger than theoretically accountable by the change in A. This excessive alteration in rheobase can be shown to explain the absence in calcium-treated nerves of the initial curvature theoretically predicted for the curve relating the relative thresholds of stimulation and the time constants of exponential current rise (from which λ is determined). The absence of this

animals moved, were intensified by a shrill note from an air hose. This stimulus caused 3 of the animals with stage 2 paralysis to run frantically around the cage for 20 to 30 seconds. The rear legs were used during this remarkable outburst of activity which terminated in collapse without convulsions or loss of consciousness.

From 3 to 10 weeks after the appearance of the first stage of paralysis the weights of the male rats (330 to 380 g) began to decline. At the end of 16 to 20 weeks they had lost 60 to 100 g. A representative weight curve is shown in Fig. 1.

Attempts to cure the paralysis in 2 of the males through the administration of 40 mg of the vitamin E concentrate per week for 16 and 20 week periods were unsuccessful. However, the progress of the neuro-muscular symptoms was definitely arrested and a growth response was elicited (Fig. 1). Doubling the carotene or methyl linolate intake or supplementing with 0.6 g of ether-extracted yeast daily failed to retard the development of symptoms or the decline in weight.



Weight curves of adult male rats reared and maintained on a low-fat vitamin E-deficient diet. Numerals indicate stage of paralysis. 7 indicates the addition to the diet of a vitamin E concentrate.

muscle pathology has also been described by Evans, Emerson and Telford.⁴ We have previously reported⁵ the prevention of gross symptoms with a vitamin E concentrate, and Knowlton, Hines and Brinkhous⁶ have demonstrated that alpha-tocopherol acetate will prevent or cure the muscle changes occurring prior to the appearance of gross symptoms.

This paper concerns the production of paralysis within 8 to 10 months in rats obtained from a stock receiving ample amounts of vitamin E, the symptomatology of these animals, and their response to a vitamin E concentrate.

Four male and 4 female rats weighing 35 g were placed on a highly purified vitamin E-deficient diet containing but 0.0056% of non-vitamin lipids.⁵ One female died at 15 weeks and one at 29 weeks of unknown causes. At 32 to 40 weeks the remaining animals developed the first stage of paralysis as manifested by a spreading of the hind legs and a marked lowering of the posterior abdominal region while walking. The weekly administration of 40 mg of a vitamin E concentrate,⁷ possessing antisterility activity in a single 3 mg dose, to one of the female rats showing the first signs of paralysis prevented the development of further symptoms during the remaining 37 weeks of the experiment.

By 45 to 50 weeks the untreated animals showed the second stage of paralysis characterized by extreme abduction of the hind legs, which were now practically useless for locomotion. In another 10 to 12 weeks the disease had become so severe that in walking the hind quarters were dragged along the floor with both legs swinging from side to side. This, the third stage of paralysis, had not developed in the untreated female by the 73rd week of the experiment.

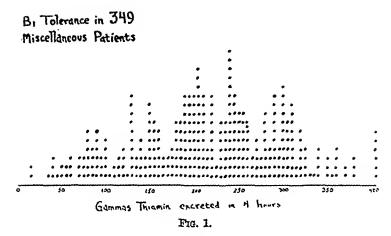
Several weeks after the second stage of paralysis the untreated animals, particularly the males, developed tremors and incoördination of the forelegs and head. So severe did these symptoms become that when eating, the rats were unable to maintain their fore feet in one position in the low food pans. The continuous jerking of the head rendered unsuccessful many of the attempts to obtain a mouthful of food. The tremors, which were most marked when the

⁴ Evans, H. M., Emerson, G. A., and Telford, I. R., Proc. Soc. Exp. Biol. and Med., 1938, 38, 625.

⁵ Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., Biochem. J., 1939, 33, 935.

c Knowlton, G. C., Hines, H. M., and Brinkhous, K. M., Proc. Soc. Exp. Biol. AND Med., 1939, 42, 804.

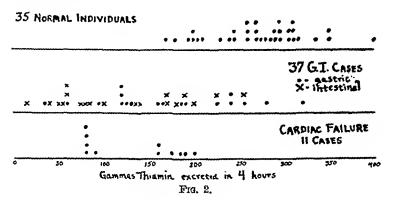
⁷ Mackenzic, C. G., Mackenzie, J. B., and McCollum, E. V., Pub. Health Rep., U. S. P. H. S., 1938, 53, 1779.



results of the test on 349 unselected patients. The distribution curve of the results indicates that an excretion of 180 gammas or more in the 4-hour period represents the average normal. Patients excreting less than 180 gammas may be considered as below average saturation.

This group of patients represents the population of hospitals and dispensaries and cannot be considered a representative cross section of the population. Fig. 2 shows the tolerance test on 35 normal, healthy students, instructors and physicians. Here it is seen that excretion values range higher. On the same figure are shown values of selected groups of patients suffering with gastro-intestinal diseases and another group in cardiac failure. The differences in range of excretion are more than significant.

Because of the known relation between carbohydrate metabolism and Vitamin B₁ a group of 132 patients with diabetes mellitus were subjected to the test. The distribution curve of the excretion values



Microscopic examination of the thigh muscles revealed lesions similar to those described by other workers.^{3, 4, 6} These lesions were not so extensive as those occurring in young rabbits with severe nutritional muscular dystrophy, a condition cured by vitamin E.⁸

Summary. Rats grown and maintained on a highly purified vitamin E-deficient diet developed paralysis of the rear legs accompanied by tremors and incoördination of the fore legs and head. Although cures could not be obtained, the administration of a vitamin E concentrate arrested the development of these symptoms and stimulated growth.

11367 P

A Test Proposed to Measure Vitamin B1 Saturation in Humans.

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A test designed to measure the individual patient's saturation with respect to Vitamin B₁ would have a wide clinical application. Experiments have been carried out in an attempt to develop such a procedure. The validity of such a test depends upon, among other things, the accuracy of the method of assay for the Vitamin B₁. The assay methods fall into two categories—chemical and biological. The chemical methods on the whole are specifically for pure thiamin. Among the biological methods the Schultz, Atkins and Fry technic which employs the rate of fermentation of glucose by a yeast is the most suitable for clinical investigation. This method measures not only the thiamin but the pyrimidines as well. The pyrimidines present in the urine may be considered for practical purposes as originating from the members of the B complex. The actual test in its present form employed in this laboratory is summarized as follows:

Patients were injected with 1 mg of thiamin hydrochloride intramuscularly in the fasting state. The urine was collected for a 4-hour period following the injection. The Vitamin B₁ activity of this collected urine was assayed by means of the Schultz, Atkins and Fry yeast fermentation method. Fig. 1 shows the tabulation of the

⁸ Mackenzie, C. G., and McCollum, E. V., J. Nutr., 1940, 19, 345.

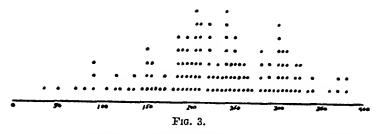
Toxicity varies with different specimens and is more concentrated in the cells and debris than in the "plasma." Whole menstrual discharge given subcutaneously in 0.1 to 1.0 cc amounts (depending upon the specimen) twice daily to normal mature females, starting when they are in preëstrus, often results in death within 48 hours. Within 24 hours or less of the first injection the animals are apprehensive and "hunched up"; their fur is ruffled; water intake increases; the nose and inner canthi of the eyes become encrusted with blood and there is firm edema of a wide area around the site of injection. Death is not ushered in by convulsions. At autopsy, the adrenals are of a dark reddish-brown color, the liver is usually dark and mottled and the lungs are congested, as compared with controls. Occasionally blood is found in the urinary bladder. Under the microscope the lungs show edema and capillary hemorrhage, the kidneys varying degrees of parenchymatous degeneration, congestion and often capillary hemorrhage. The liver shows some degeneration. The most consistent picture is seen in the adrenal cortex, diffuse or focal hemorrhage and dissolution of cells in either or all zones. the adrenal cortices of rats that have died early, hemorrhage may be the only finding. With increased length of survival, dissolution to actual necrosis of cells is also seen, along with increased vacuolization.

Animals in which injections are started during the beginning of postestrus are most likely to survive and become comparatively resistant to continued injections. On exploration after the third or fourth day, their ovaries contain large, red corpora lutea. The subcutaneous edema becomes replaced by brawny induration, so that in less than 15 days the stiffness and fixation of the pelt prohibit further injections.

If each dose of menstrual discharge is accompanied by the subcutaneous injection of 1 to 2 r.u. of a native estrogen (we usually have used $0.05 \, \gamma$ of estradiol), the animals almost invariably die within 48 hours of the first injections, regardless of the time of the cycle at which administration is started. Clear "plasma" from the discharge is usually innocuous in normal mature female rats in fairly large amounts (1 to 2 cc twice daily) at any stage of the cycle unless estrogen is concomitantly given, in which case the typical rapid death ensues. In control experiments with venous blood or serum with or without estrogen, the only deleterious effect noted was subcutaneous induration when more than 2 cc of whole venous blood was administered daily.

The most consistently lethal effect has been noted in 19- to 24-

Diabetes Mellitus 132 patients



so closely parallels that of the larger unselected group of patients that one can infer no particular unsaturation of Vitamin B₁ in clinical diabetes mellitus

11368

Menstrual Discharge of Women. I. Its Toxicity in Rats.*

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In an effort to find a factor to which the local processes resulting in menstruation might be attributable, 37 specimens of menstrual discharge, each varying from 30 to 120 cc in amount, donated by 5 normally menstruating, parous women, have been studied. They have been collected by means of soft rubber cups.† During collection each portion has been placed in the refrigerator immediately upon removal. For the most part, the experiments to be reported have been performed upon the whole specimen after pooling the various portions. For control experiments, whole venous blood, drawn during the first day of menstruation and citrated or mixed with sufficient distilled water to prevent clotting and kept in the refrigerator, has been used in similar or larger amounts.

Menstrual discharge has been found to be highly toxic to rats, their resistance being markedly affected by hormonal conditions.

^{*} The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy, aided by grants from the Committee for Research in Problems of Sex, National Research Council.

[†] The Hy-Kup Distributors (National), Indianapolis, Indiana.

estrogen be given with the discharge. Chorionic gonadotropin does not save mature females from the combined injection of discharge and estrogen. Desoxycorticosterone acetate (10 to 20 mg) does not prevent death from a M.L.D. of discharge in immature females or from discharge plus estrogen in mature females even when its administration is begun 2 days before injecting the toxin. Adrenal cortical extract (Eschatin, P. D. & Co.) in 1-cc doses twice daily for 2 days before and during the day of injection of twice the M.L.D. of discharge completely protected an immature female; a littermate control receiving the same amount of the same discharge on the same day died in 20 hours.

Immature and mature female rats may be rendered resistant to several times the M.L.D. of discharge alone or discharge plus estrogen by injection of sublethal doses twice a week for 2 to 3 weeks. Such immunity has been maintained for as long as 4 months. Mature female rabbits are extremely susceptible to the discharge alone, 1 to 3 subcutaneous injections of 1 cc usually resulting in death within 48 hours. By extreme caution, 1 rabbit was made to survive 14 days of small injections. Its serum, in 5 1-cc amounts over 2½ days prior to the injection of twice the M.L.D. of a specimen, completely protected an immature female rat. A pseudoglobulin fraction of this same serum also protected immature female rats against lethal doses of 2 other samples of discharge from 2 different donors.

Only by preliminary treatment were we able to protect rats against the menstrual toxin with progesterone, adrenal cortical extract or immune serum. After the discharge had been administered, even massive amounts of any of these failed to prolong the period of survival.

In an attempt to determine whether the toxicity of menstrual discharge is due to a specific toxin or simply to products of bacterial action or protein decomposition, the following experiments were run. Sterile citrated whole venous blood was incubated for 48 hours after inoculation with discharge. This material was toxic to immature rats but not lethal, even in amounts 3 to 5 times the M.L.D. of fresh discharge. The striking edema which characterizes the reaction to menstrual discharge was lacking in rats injected with inoculated venous blood. Furthermore, it was found that sterile venous blood alone, after 48 hours at incubation temperature, is as toxic as inoculated material. These experiments, therefore, gave no conclusive evidence either for or against the specificity of menstrual toxin. Discharge collected on the fourth day of flow in a sterile cup following a douche with 2 quarts of water killed an

day-old female rats. Thirty-five of the 37 specimens so tested have produced death, when given twice daily, in less than 48 hours from the first injection, the total dose varying with different specimens between 0.01 and 0.8 cc. The only 2 specimens non-lethal in 0.8-cc amounts in immature females were from the same donor and had been collected during the first day of the period when flow was profuse and contained little or no debris. In this individual, specimens during the last days of flow contained much brown debris and were very much more toxic, a single injection of 0.01 to 0.1 cc being usually lethal to immature female rats. Two to 4 cc of whole venous blood in 4 doses over 2 days is easily tolerated by immature female rats. Spayed immature female rats, and male rats, both immature, mature and castrated mature, have a relatively greater resistance to the discharge, and mature females, spayed more than 4 weeks previously, have tolerated even larger amounts. ministration of estrogen to spayed females does not render them more susceptible to the discharge. In 2 experiments, a mash of 6 to 7 fresh mature rat ovaries injected simultaneously with each dose of the discharge and estrogen produced the typical rapid death in spayed females; the control spayed females receiving the same amounts of the same discharge and estrogen survived.

From these observations, it would appear that the greatest susceptibility to the menstrual toxin depends upon the presence of the ovaries and that corpora lutea afford partial protection, but that the administration of estrogen overrides this protective action.

The toxicity of the discharge is destroyed by heat, ethyl alcohol, acetone or acid, in amounts sufficient to precipitate the proteins. It is diminished by raising the pH to over 8 or by allowing the specimens to become putrid either by standing at room temperature for a few days or in the refrigerator for at least 3 weeks. Dried rapidly in vacuo over CaCl₂, powdered, sealed and refrigerated, the material retains its toxicity for at least 8 months. The toxin is not soluble in the usual lipoid solvents and is nondialysable. After fractional precipitation with (NH₄)₂SO₄, it is found in greatest concentration in the water insoluble portion (after dialysis) of the englobulin precipitate.

Mature female rats have been completely protected against a lethal dose of menstrual discharge plus estrogen by the preliminary and simultaneous administration of large amounts of progesterone, to a total of 15 to 30 mg. Chorionic gonadotropin, if given so as to produce a good luteinizing response by the time a M.L.D. of the discharge is injected, protects immature female rats, but not if

to normal cycles when injections were discontinued. One to 2 cc twice daily of either whole menstrual discharge or "plasma" produce this effect, but "plasma" (without estrogen) is less likely to be lethal and is not as irritating to the subcutaneous tissues. Exploration revealed large corpora lutea which were functional, as demonstrated by the finding of deciduomata on the ninth day of injections following needling of the uterus on the sixth day.

Such a progesterone-stimulating action suggested that the discharge might contain either a known gonadotropic hormone or an estrogen, although the familiar estrogens in amounts sufficient to stimulate demonstrable hyperactivity of the corpora would first produce estrous vaginal smears. In searching for estrogenic potency, we have extracted large amounts of whole menstrual discharge, "plasma" and dried whole discharge with ethyl alcohol, ether, acetone, butyl alcohol and benzene. We have also attempted to recover any combined estrogen by performing acid hydrolysis upon wet and dried whole discharge and butyl extracts of the same. None of these preparations, in olive oil solution or saline suspension, has given estrus in spayed mature female rats, standardized for estrogen assay, even when tested upon a number of primed animals for as little as 1 r.u. in 20 cc of material. Although Frank³ has reported estrogenic activity in menstrual discharge, our results demonstrate that, if present at all, there is not enough to account for the increased production of progesterone in normal mature rats. These lipoid extracts have neither been toxic nor had any effect upon the cycles of rats.

In searching for gonadotropic substance, whole discharge was shaken with 5 volumes of 95% ethyl alcohol and placed in the refrigerator overnight. After centrifugation, the precipitate was washed twice with ether, dried, powdered and taken up in water. After 24 hours in the refrigerator, with repeated shaking, the water-

t Crystalline estrone in water is rapidly destroyed by acid hydrolysis methods which may be used upon urine without loss of added estrone. A solution of high salt concentration (69 g NaH₂PO₄ and 179 g Na₂HPO₄ per L) has been found a satisfactory substitute for urine and used as the medium for hydrolysis in attempting to deconjugate any combined estrogens in menstrual discharge or extracts of it. The material to be tested has been diluted with 10 volumes of concentrated phosphate solution, boiled under a reflux for 10 minutes with 15 vol.% HCl² and extracted for 24 hours in a continuous benzene extractor.

² Smith, O. W., Smith, G. V., and Schiller, S., Endocrinol., 1939, 25, 509.

[‡] A total of 22 lipoidal extracts of whole menstrual discharge or plasma have been tested, each representing from 40 to 270 cc of material.

³ Frank, R., The Female Sex Hormones, Charles C. Thomas, published 1929.

immature female rat in 30 hours. This material was given in a single injection of 0.25 cc within 40 minutes from the time it passed the cervix. Its toxicity would seem to rule out bacterial activity or any protein decomposition other than what might occur in the uterus. The strongest indication of a specific toxin lies in the repeatedly confirmed observation that 1 to 2 cc twice daily of specimens of whole discharge or "plasma," which alone in these amounts are non-toxic to normal mature female rats, are lethal within 72 hours from the first injection when estrogen is simultaneously administered, whereas such is not the case with venous whole blood or serum.

Conclusion. The menstrual discharge of women with normal cycles is highly toxic to rats through the production of vascular damage. The possibility that this toxicity is accountable to protein decomposition has not been conclusively ruled out, although the marked effect of hormonal conditions upon resistance appears to argue against this. The greatest susceptibility requires the presence of the ovaries. Functional corpora lutea afford partial protection, but the administration of estrogen overrides this action. Protection may be rendered by pretreatment with large amounts of progesterone, adrenal cortical extract or "immune" rabbit serum. The toxin appears to be intimately associated with a large moleculed protein material.

11369 P

Menstrual Discharge of Women. II. Its Progesterone-Stimulating Effect in Mature Rats.*

O. Watkins Smith and George Van S. Smith.

From the Fearing Research Laboratory, Free Hospitol for Women, Brookline, Mass.

Early in the study of the toxicity of the catamenial discharge¹ it was noted that mature female rats, with previously regular cycles, which survived the first 2 or 3 days of injections went, within 72 hours, into constant diestrus on continued injections and reverted

1 Smith, O. W., and Smith, G. V., PROC. Soc. Exp. Biol. and Med., 1940,

44, 100.

^{*} The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy, aided by grants from the Committee for Research in Problems of Sex, National Research Council.

Conclusion. The menstrual discharge of normally menstruating women contains a heat-stable factor, insoluble in lipoid solvents, which stimulates increased luteal activity in mature rats. This factor has also been found in venous blood and urine at the time of menstruation. Its progesterone-stimulating effect is not accountable to any free or combined estrogen or to any known gonadotropic hormone.

So far as this work has progressed, the progesterone-stimulating factor of menstrual discharge resembles the substance reported by Astwood and Greep as occurring in the rat placenta. Our material has not yet been tested upon hypophysectomized rats but the fact that alcohol precipitation destroys toxicity without decreasing progesterone-stimulating activity makes it appear that the progesterone stimulation is not a non-specific toxic effect.

11370

Unsuccessful Therapy in Experimental Equine Encephalomyelitis with Salt Solutions of Varied Concentrations and Sulfanilamide Compounds.*

J. EMERSON KEMPF AND MALCOLM H. SOULE.
From the Hygienic Laboratory, University of Michigan, Ann Arbor.

The high mortality of Eastern equine encephalomyelitis in both man and animals has prompted interest in therapy. Specific antisera may have value in the treatment of horses if administered early, but its use after the disease is well established is ineffectual. In horses the disease may be suspected and treated specifically; however, in man the diagnosis must usually await the appearance of neurological signs at which stage the lesions are too far advanced for antiserum therapy. This shortcoming suggested the investigation of other therapeutic procedures employing the highly susceptible rat and mouse.

The beneficial effect of hypertonic solutions, such as 10-25%

⁴ Astwood, E. B., and Greep, R. O., Proc. Soc. Exp. Biol. and Med., 1938, 38, 713.

^{*} This work was aided by a grant from the Clara Ward Seabury Clinic for Infantile Paralysis.

¹ Personal communication, Dr. B. M. Lyon, Assistant Director, Vet. Dept., Lederle Labs., New York.

insoluble residue was separated by centrifugation, washed 3 times with water and discarded, the watery extract (with washings) being made up to a measured volume and used for testing. Such extraction appears to yield a complete recovery of the progesteronestimulating principle, since, with 12 specimens from 3 donors, the equivalent of 1 cc of discharge twice daily for 8 days was sufficient to increase and prolong the activity of corpora lutea in mature rats, with the production of deciduomata after needling of the uterus on the sixth day. These extracts have shown no toxicity other than a slight subcutaneous reaction. As tested on spayed rats, they contained no free or combined estrogen.† They have had no effect upon the genitalia of immature female rats in amounts equivalent to 6 cc of whole discharge. The combined injection of one of them and F.S.H. (from menopausal urine) into an immature female rat gave no luteinization. They have not enhanced the luteal response of immature rats to chorionic gonadotropin and have repeatedly failed to augment the weight of the seminal vesicles of immature males (the equivalent of 10 cc of material being given in 10 doses over 5 days). These findings rule out the presence of any known follicle-stimulating or luteinizing hormone. Furthermore, activity was not diminished by heating in a boiling water bath for 1 hour, which treatment is destructive of the known gonadotropic hormones.

After fractionation of the proteins of menstrual discharge with (NH₄)₂SO₄, the progesterone-stimulating activity is recovered in the water-insoluble portion (after dialysis) of the englobulin precipitate. The toxicity of menstrual discharge is also recovered in greatest concentration in this fraction. § The toxin and progesteronestimulating substance are not identical, however, since alcohol precipitation destroys the former but not the latter. Furthermore, the latter has been found in nonlethal materials, such as alcohol-ether precipitates of venous blood and urine at the time of menstruation and in a sample of catamenial discharge that had lost its toxicity on standing. None has been demonstrable in venous blood, in the amounts tested, during the luteal phase of the cycle or in blood from males. In testing for it, upon normal mature rats with previously regular cycles, the result is considered positive only when deciduomata, confirmed by microscopic section, are present on the eighth or ninth day of injections after needling of the uterus on the fifth or sixth day.

 $[\]delta$ These findings suggest that the progesterone-stimulating factor occurs in menstrual discharge in a toxic protein conjugation which is split by alcohol but not by $(NH_4)_2SO_4$ precipitation.

2-sulfanilamidothiazol (sulfathiazol), 2-sulfanilamidomethylthiazol (sulfamethylthiazol), and 2-sulfanilamidophenylthiazol (sulfaphenylthiazol). The animals were inoculated intracerebrally. Treatment with the drugs was started 24 hours later and was continued twice daily by the intraabdominal route. The rats received 40 mg of sulfanilamide (human dose of sulfanilamide compounds: maximum 6 g daily per os); 2 mg of sulfapyridine, and mg of the thiazol compounds. The 5 sulfanilamide compounds were injected into the mice in 2 mg doses following the aforementioned technic. This procedure is open to criticism based on the clinical opinion that in order to maintain a satisfactory blood level of the drug it must be given 4 times daily. However, in the hands of Barlow and Homburger⁹ the treatment of staphylococcus infections of mice with 2 daily doses of thiazol compounds administered by stomach tube was successful. In addition, 1,000 times the minimal cerebral lethal dose may be considered a too severe inoculum, but in man the virus would be present in high con-

TABLE II. Chemotherapy of EEE in Rats Infected with 1,000 MCLD.*

Treatmen	it				
Compound	Dose, mg twice daily	No. of rats	Mortality		
None (control) Sulfanilamide Sulfapyridine Sulfathiazol Sulfamethylthiazol Sulfaphenylthiazol	40 2 15 15 15	16 14 14 12 9	% 16 (100) 14 (100) 14 (100) 12 (100) 8 (89) 9 (100)		

^{*}See footnote Table I.

TABLE III. Chemotherapy of EEE in Mice Infected with 1,000 MCLD.*

Treatmen	ıt				
Compound	Dose, mg twice daily	No. of rats	Mortality		
None (control) Sulfanilamide Sulfapyridine Sulfathiazol Sulfathethiazol Sulfaphenylthiazol	2 2 2 2 2 2	12 12 12 12 12 12 12	76 12 (100) 12 (100) 11 (92) 11 (92) 10 (83) 12 (100)		

^{*}See footnote Table I.

⁹ Barlow, O. W., and Homburger, E., Proc. Soc. Exp. Biol. and Med., 1939, 42, 792.

glucose, in edema of the CNS has long been recognized, particularly in edema of traumatic origin. In contrast, poliomyelitis of man and monkey has apparently responded favorably to injections of hypotonic solutions in the hands of Retan.² Therefore, the effects of hypertonic and hypotonic saline solutions were compared in rats with experimental EEE using isotonic saline as a control. Intraabdominal injections of the solutions were begun 40 hours after intracranial inoculation of the rats, at which time about 10% of the animals showed well advanced signs of the disease. The remaining rats appeared normal. The fluids were administered in 4 cc quantities every 2 or 3 hours, totaling approximately 40 cc daily for a 150 g rat. This is equivalent to 12 liters for a 60 kg man. The saline injections were continued until 83% of the animals were dead. This treatment in no way altered the course of the infection (Table I).

Many workers have reported the use of sulfanilamide and related compounds on virus infections of man and animals. In man sulfanilamides seem to be of value in lymphopathia venereum,³ and in animals success has followed the treatment of meningo-encephalitis associated with canine distemper,⁴ with negative results in poliomyelitis,⁵, ⁶, ⁷ rabbit myxoma, rabbit fibroma, herpetic encephalitis, choriomeningitis and St. Louis encephalitis.⁸

The following compounds were selected for evaluation in the treatment of EEE in rats and mice: sulfanilamide, sulfapyridine,

TABLE I.

Effect of Intraäbdominal Injections of Saline Solutions of Varied Concentrations on EEE in Rats Infected with 10 MCLD.*†

Tre				
Solution	Dose, cc	Frequency	No. of rats	Mortality
				%
None (control)			45	36 (80)
Hypertonic Saline-3%	4	Every 2-3 hr	45	39 (86)
Hypotonic Saline-0.375	% 4	,, ,,	45	39 (86)
Isotonic Saline-0.875%	4	" "	45	36 (80)

^{*}Duration of the experiment was 72 hr. †MCLD = Minimal cerebral lethal doses.

² Retan, G. M., J. Ped., 1937, 11, 647.

³ Shaffer, L. W., and Arnold, E., Arch. Derm. and Syph., 1938, 38, 705.

⁴ Marcus, P. M., and Nechcles, H., Proc. Soc. Exp. Biol. AND Med., 1938, 38. 385.

⁵ Toomey, J. A., and Takacs, W. S., Arch. Ped., 1938, 55, 307.

⁶ Kelsen, S. R., Proc. Soc. Exp. Biol. and Med., 1937, 36, 718.

⁷ Toomey, J. A., and Takacs, W. S., Arch. Ped., 1939, 56, 384.

⁸ McKinley, E. B., Meck, J. S., and Acree, E. G., J. Infect. Dis., 1939, 64, 36.

occurs after inoculation of 10⁻² mg tubercle bacilli, but either medium will produce profuse growth after inoculation of 10⁻⁶ mg bacilli if 5% unheated serum is added to the medium.

The total lipoids extracted from the serum by alcohol and ether are inhibiting and the phospho-lipins are without effect in contrast to what was observed in egg yolk. When the proteins are precipitated by alcohol and redissolved in distilled water, they have the same growth-promoting effect as the original serum, while the filtrate (after removal of the alcohol) is inhibiting.

Separation of the serum proteins in albumen and globulin by half saturation with ammonium-sulfate, showed the albumen fraction to be strongly growth-promoting (after dialysis), while the globulin fraction was inhibiting or without effect. Crystalline horse serum albumen after three recrystallizations was strongly growth-promoting when added in a concentration of 0.1% to synthetic medium; rapid and abundant growth occurred after inoculation of as little as 10^{-7} mg tubercle bacilli. Autoclaving for 10 minutes at 115° C of the synthetic medium containing the serum albumen destroys the growth-promoting effect.

Horse serum albumen after 3 recrystallizations still contains numerous impurities, amongst which are globulins, enzymes, pigments and flavoprotein. Experiments are now in progress to prepare a horse serum albumen completely free of such admixtures.

The growth-promoting activity of crystalline horse serum albumen is much greater than that of the lipoid extracts of egg yolk previously described. Addition of 0.3% egg yolk gave good growth

No. of Colonies of Tubercle Bacilli Developing in Depth of Mcdia After Inoculation of Varying Amounts of Bacilli.

Mg tubercle bacilli inoculated	Glycine medium	Asparagine- ammonium citrate medium	Glycine medium with 0.1% crystalline horse serum albumen	Asparagine- ammonium citrate medium with 0.1% crystalline horse serum albumen
10-1	7	innumerable	innumerable	innumerable
10-2	0	8	7.5	* *
10~3	0	3	3.3	2.5
10-4	Ô	ō	23	>>
10-5	Ō	Õ	22	"
10-6	Ö	0	80	>>
10-7	0	0	6	30
10-8	0	0	0	1

⁶ Boissevain, C. H., and Schultz, H. W., Am. Rev. Tuberculosis, 1938, 38, 624.

centration before the diagnosis could be made and the drug administered.

The data are presented in Tables II and III. The drugs under the conditions of the experiment had no apparent effect on the course of the infection.

11371 P

Growth Promotion of the Tubercle Bacillus by Serum Albumen.

C. H. Boissevain.

From the Laboratory of the Colorado Foundation for Research in Tuberculosis at Colorado College, Colorado Springs, Colo.

The author' described the growth of single colonies of tubercle bacilli in the depth of coagulated rabbit plasma. It was then observed that rabbit serum + agar was a less favorable medium than coagulated plasma. Only a few colonies developed in "hormone agar", but many more in hormone agar + rabbit serum. The growth-promoting effect of serum was quite variable. Evans and Hanks' confirmed the favorable effect of rabbit serum and obtained good growth in the depth of Long's medium after the addition of blood or serum. Kallo and Nathan's and Pagel' found that some human sera support the growth of tubercle bacilli while others fail to do so. Pagel could not demonstrate the presence of either growth-promoting or specific inhibiting substances. Dreas confirmed the inhibiting effect of agar and found that tubercle bacilli grew in the depth of a modified Long's medium when it had been inoculated with varying quantities of a bacillary emulsion.

The addition of human, guinea pig, rabbit, sheep or horse serum all enhance the growth of tubercle bacilli in the depth of synthetic medium. The growth appears earlier, is more abundant and takes place after inoculation of smaller quantities. In synthetic medium where the nitrogen is supplied by glycine, growth rarely occurs after inoculation of less than 10⁻¹ mg tubercle bacilli, in media with asparagine-ammonium citrate as nitrogen source, growth frequently

¹ Boissevain, C. H., Am. Rcv. Tuberculosis, 1926, 18, 90.

² Evans, B., and Hanks, J. H., PROC. Soc. EXP. BIOL. AND MED., 1939, 40, 112.

³ Kallos, P., and Nathan, E., Z. f. Immunitäts forschung, 1932, 76, 393.

⁴ Pagel, W., Tubercle, 1934-35, 16, 256; J. Path. and Bact., 1940, 50, 111.

⁵ Drea, W. F., J. Bact., 1940, 39, 197.

occurs after inoculation of 10⁻³ mg tubercle bacilli, but either medium will produce profuse growth after inoculation of 10⁻⁶ mg bacilli if 5% unheated serum is added to the medium.

The total lipoids extracted from the serum by alcohol and ether are inhibiting and the phospho-lipins are without effect in contrast to what was observed in egg yolk.^a When the proteins are precipitated by alcohol and redissolved in distilled water, they have the same growth-promoting effect as the original serum, while the filtrate (after removal of the alcohol) is inhibiting.

Separation of the serum proteins in albumen and globulin by half saturation with ammonium-sulfate, showed the albumen fraction to be strongly growth-promoting (after dialysis), while the globulin fraction was inhibiting or without effect. Crystalline horse serum albumen after three recrystallizations was strongly growth-promoting when added in a concentration of 0.1% to synthetic medium; rapid and abundant growth occurred after inoculation of as little as 10^{-7} mg tubercle bacilli. Autoclaving for 10 minutes at 115° C of the synthetic medium containing the serum albumen destroys the growth-promoting effect.

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TABLE I.

No. of Colonies of Tubercle Bacilli Developing in Depth of Media After Inoculation of Varying Amounts of Bacilli.

Mg tubercle bacilli inoculated	Glycine medium	Asparagine- ammonium citrate medium	Glycine medium with 0.1% crystalline horse serum albumen	Asparagine- ammonium citrate medium with 0.1% crystalline horse serum albumen
10-1	7	innumerable	innumerable	innumerable
10-2	0	8	27	11
10-3	0	3	11	* *
10-4	0	ā	"	"
10-5	0	ñ	2.2	1)
10~6	0	Ŏ	80	,,
10~7	0	Ö	6	30
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The serum was fractionated with the aid of the somewhat modified method of Parsons, originally recommended for crystallization of hemoglobins and in these experiments consisting of repeated alternate freezing to -14°C and centrifugalization of the serum at 4°C until complete thawing. The procedure enabled the separation of colorless, faintly colored, medium colored, and dark red fractions. Small amounts of crystals and whitish debris were also obtained.

Following determinations of the concentration of hemoglobin by the Sahli method modified by Wintrobe (Hellige haemometer), all the materials were promptly injected into guinea pigs weighing each 350 g. The volume injected into each guinea pig ranged between 1 cc-10 cc and never exceeded 10 cc. One cc was given subcutaneously over the abdomen, the remaining amount was introduced subcutaneously over both thighs in equally divided doses. Examinations for symptoms of generalized and local tetanus were made daily. Test guinea pigs recorded as having shown tetanus intoxication died following generalized paralysis and spasms. On postmortem exmination no other pathological findings were observed except localized inflammation at the site of injection without pus formation, and some congestion of lungs and adrenals. The results of the tests were consistently as follows:

The blood defibrinated by means of glass beads elicited tetanus.

The washed red blood cells, the cellular debris and packed hemoglobin crystals suspended in a small amount of 0.85% NaCl solution failed to produce the symptoms.

Laked red blood cells tested before centrifugalization, and the clear dark red supernatant fluid obtained after centrifugalization of the laked cells, both possessed a considerable amount of tetanus toxin, which in sufficient concentration produced acute paralysis and death within 48 hours following the subcutaneous injection.

The plasma containing a certain amount of hemoglobin also had the tetanospasmin. Fractionation proved, however, that the major portion of the toxin was associated with the dark red portion; a small amount capable of producing only local tetanus being present in the medium-colored fraction, and none at all found in the colorless and slightly colored parts.

Quantitative studies emphasize the remarkable fact that under the above experimental conditions the tetanospasmin concentration of the blood is approximately directly proportional to the hemoglobin concentration. With doses 20-40 M.L.D. of the toxin injected sub-

¹ Parsons, quoted in The Respiratory Function of the Blood... Part II. Hemoglobin, p. 68, by Joseph Barcroft, Cambridge. University Press, 1928.

after planting 10⁻⁵ mg bacilli while 0.1% horse serum albumen supports growth after planting 10⁻⁷ mg bacilli.

Summary. Crystalline horse serum albumen was shown to be strongly growth-promoting for tubercle bacilli grown in synthetic media. Horse serum globulins were without effect or inhibited growth.

11372 P

Association of Tetanospasmin with Hemoglobin in Acute Stages of Tetanus Intoxication of Guinea Pigs.

GREGORY SHWARTZMAN.

From the Laboratories of The Mount Sinai Rospital, New York, N. Y.

The object of this communication is to report on a new observation that tetanus toxin (tetanospasmin) may be found intimately associated with the hemoglobin of guinea pigs during the paralytic stages of the disease. A part of an extensive series of experiments on this problem is briefly summarized, as follows:

Guinea pigs weighing each 350 g were injected subcutaneously in the abdominal region with tetanus toxin* in doses ranging between 20 to 40 M.L.D. and bled from the heart during the stages of complete paralysis and spasmodic contractions (i.e., 24-48 hours following the subcutaneous injection). The pooled blood was defibrinated by shaking with glass beads; filtered through several layers of gauze, and centrifuged at room temperature for one-half hour. The clear, dark red supernatant plasma and the sedimented red blood cells were placed in separate containers.

Washing of red blood cells in 1% NaCl solution was repeated until the supernatant fluid became biuret-negative (usually 4 washings are required). The washed cells were diluted 1:5 in distilled water. When fairly complete laking and some crystallization occurred about one hour later, the solution was centrifuged for one-half hour also at room temperature. This procedure resulted in separation of a dense dark red sediment containing masses of hemoglobin crystals; a superimposed loose whitish precipitate consisting of cellular debris and some hemoglobin crystals; and finally, a clear dark red supernatant fluid.

^{*} Tetanus toxin in powder form obtained from Eli Lilly Research Laboratories (Lilly 27994, 400,000 M.L.D. per g), through the courtesy of Dr. H. M. Powell.

The serum was fractionated with the aid of the somewhat modified method of Parsons, originally recommended for crystallization of hemoglobins and in these experiments consisting of repeated alternate freezing to -14°C and centrifugalization of the serum at 4°C until complete thawing. The procedure enabled the separation of colorless, faintly colored, medium colored, and dark red fractions. Small amounts of crystals and whitish debris were also obtained.

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11373 P

Association of Meningococcus and B. typhosus Toxins with Hemoglobin in vitro.

GREGORY SHWARTZMAN.

From the Laboratories of The Mount Sinai Hospital, New York, N. Y.

Incidental to the observations that a close association exists between tetanospasmin and hemoglobin in the blood of guinea pigs suffering from tetanus intoxication¹ a series of experiments was carried out on combination of hemoglobin with bacterial toxins in vitro. The results are briefly presented in this communication.

The toxins employed were filtrates from the "agar washings" of cultures of meningococcus and B. typhosus which were highly potent in the elicitation of the phenomenon of local skin reactivity. In this work they were purified by dialysis in cellophane bags No. 600 against 0.85% NaCl solution for a period of one week.

The hemoglobin preparations were made in the following manner: All the work was done under strict precautions of sterility and each step controlled for bacterial contamination on aerobic and anaerobic media. Rabbit blood obtained from the heart was defibrinated by shaking with glass beads, filtered through several layers of gauze and centrifuged in order to separate the plasma from the erythrocytes. The erythrocytes were promptly washed in 1% NaCl solution by repeated centrifugalization until the washings became biuret-negative (from 5-7 washings being required). The packed red blood cells were kept frozen for several hours at -70°C in a mixture of cellosolve and dry ice and gradually thawed out in a mixture of ice and alcohol and in the refrigerator at 4°C overnight. The cells were diluted in distilled water to a hemoglobin concentration of 50-60% as determined in the Hellige haemometer.

Filtration of the solution through a Seitz filter resulted in effective removal of cellular debris, as ascertained by examination of spreads stained by Wright's method and hanging-drop preparations. In control experiments adjusting the pH to 5.5 by the addition of N/10 HCl gave no precipitate in the filtrates.* The clear, dark red

¹ Shwartzman, G., Proc. Soc. Exp. Biol. and Med., 1940, 44, 112.

² Shwartzman, G., Phenomenon of Local Tissue Reactivity and Its Immunological, Pathological and Clinical Significance, Paul B. Hoeber, Inc., Medical Book Department of Harper and Brothers, New York, 1937.

³ Shwarizman, G., Morell, S., and Sobotka, H., J. Exp. Med., 1937, 65, 323.

⁴ Jorpes, E., Biochem. J., 1932, 26, 1488.

cutaneously per guinea pig, weighing 350 g, each M.L.D. recovered from the blood of the intoxicated guinea pigs corresponded to 0.035 g-0.007 g of hemoglobin in the preparations and fractions tested, in volumes ranging between 1 cc-10 cc. The effects of these total amounts of hemoglobin were the same, irrespective of the volumes used.

The active blood preparations may be considered predominantly oxylhemoglobin. Occasional batches of methemoglobin obtained after prolonged storage and reduced hemoglobin were not studied in this series.

No symptoms were elicited in test-guinea pigs with blood fractions of control guinea pigs receiving no injections and of those injected with tetanus toxin heated at 80°C for ½ hour prior to the injection. Also, blood fractions of guinea pigs which received an old inactive toxin gave no tetanus.

In this and the following paper the term "hemoglobin" refers to complex substances obtained in solution following laking of washed erythrocytes and removal of cellular debris. Hemoglobin is defined, therefore, by the biological properties, its chemical relationships being disregarded for the moment. The term "association" is merely descriptive and indicates a combination which may either occur directly with the hemoglobin or through the intermediary of some unidentified constituent with which the hemoglobin is associated.

All the data considered together, especially observations on the toxic activity of hemoglobin obtained by means of lysis of washed red blood cells, indicate clearly that under the experimental conditions described, a close association exists between hemoglobin and tetanospasmin in the blood recovered from guinea pigs during the acute stages of the disease.

Investigations on association of certain viruses with hemoglobin are under progress.

11373 P

Association of Meningococcus and B. typhosus Toxins with Hemoglobin in vitro.

GREGORY SHWARTZMAN.

From the Laboratories of The Mount Sinai Hospital, New York, N. Y.

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filtrates were dialyzed at 4°C for a period of several days in cellophane bags No. 600 against distilled water. The water was changed daily. The materials removed from the bags were dried in vacuo by the methods of Flosdorf and Mudd. They subsequently yielded perfect solution on addition of a diluent up to the initial volume, i.c., distilled water, Sörensen phosphate buffer solution of pH 6.8, plain broth or blood scrum.

In the experimental work about to be described an amount of dry hemoglobin representing a yield of 0.85 g of dissolved hemoglobin was mixed with various amounts of undiluted dialyzed toxins and also toxins diluted in different diluents or in normal rabbit scrum. The phenomenon-producing potency of the final concentration of toxins per one ce ranged between 50-100 reacting units. The mixtures were dried in vacuo in the Flosdorf-Mudd apparatus. or several days later the dry materials were dissolved in distilled water in amounts equal to the initial volumes of hemoglobin solutions or in phosphate buffer of pH 6.8 of the same or greater volumes. The solutions were then fractionated by the method of Parsons.⁵ After 7-10 repetitions of alternate freezings at -14°C and centrifugalizations at 4°C until complete thawing, fractions widely differing in hemoglobin concentration were obtained.

The materials were assayed for toxic activity by means of the phenomenon of local skin reactivity. In these tests, the rabbits were prepared by a single intradermal injection of dialyzed meningococcus of B. typhosus toxin (i.e., the same as used for combination with hemoglobin) and 24 hours later injected intravenously with the materials tested, one or more groups of 2 rabbits serving for each material. The results of the experiments carried out in this manner were as follows:

Rabbit, cow, guinea pig and sheep hemoglobin containing no toxin and fractionated by the method of Parsons gave no reactions

in prepared rabbits.

Hemoglobin held meningococcus and B. typhosus toxins, the activity being associated with colored fractions and totally absent from slightly colored and colorless portions. Quantitatively, toxicity was not found in hemoglobin concentrations lower than 0.012 g per one cc. The association with the hemoglobin was firm. Elution of the toxin could not be obtained either by dilution of the toxinhemoglobin combination in 3-fold larger amounts of the initial diluent, or by the use of plain broth, phosphate buffer mixture of

⁵ Parsons, quoted in The Respiratory Function of the Blood. Part II. Hemoglobin, p. 68, by Joseph Barcroft, Cambridge, University Press, 1928.

pH 6.8 and normal horse serum. The same firm association of toxin with hemoglobin was obtained when toxin mixed with normal horse serum was used for the combination. Further fractionations and elutions were done under aerobic conditions. The preparations in solution appeared of bright red color of oxyhemoglobin. Occasionally, dark brown preparations of methemoglobin were encountered. They were not studied in this series of experiments. The relation of oxidation and reduction to the association described will be embodied subsequently in a publication dealing with crystallized hemoglobin preparations.

Thus, it may be concluded from the experiments cited that hemoglobin is capable of entering into association with bacterial toxins in vitro. The combination appears firm since elution by greater dilution in water and by dilution in other diluents, as well as normal

rabbit serum, is unsuccessful.

The stable association of toxic agents in vivo and in vitro with hemoglobin suggests studies on the effects of disease-producing agents upon intimate processes of cellular physiology in which hemoglobin and similar respiratory enzymes play an important rôle.

11374 P

Regeneration of Euplanaria Dorotocephala with Pituitary Gland Extract.*

THEODORE T. BLUMBERG. (Introduced by John A. Kolmer.)

From the Biological Laboratories, Temple University, Philadelphia.

The literature presents few examples of effects upon invertebrate material by vertebrate hormones.^{1, 2} This preliminary paper reports the accelerated regeneration in posterior and anterior portions of *Euplanaria dorotocephala* in media of beef pituitary extracts. Wulzen⁸ reports the effect of feeding of ox pituitary gland upon the growth and fission of *Planaria maculata*. In the present study there was no normal feeding, for the pituitary extracts were introduced in solution and the regenerating, transected flatworms decreased in size during the experiments.

^{*} The author wishes to acknowledge indebtedness to Dr. E. J. Larson, Temple University, for his kind interest and direction.

¹ Ashbel, Rivka, Nature (London), 1935, 185, 343.

² Coldwater, K. B., J. Exp. Zool., 1933, 65, 43.

³ Wulzen, Rosalind, J. Biol. Chem., 1916, 25, 625.

The animals were cut transversely midway between the eyespots and proboseis and each part placed in a separate section dish containing 10 ml of spring water. The total number of regenerating pieces in the preliminary experiments was 112. Of these, 54 were retained as controls. Extract of whole beef pituitary in various amounts was added to the spring water in the remaining dishes, each containing a regenerating animal. The pituitary extract was prepared as follows: One gram of desiccated whole beef pituitary was added to 10 ml of spring water. The mixture was shaken, placed in a refrigerator for 24 hours, then centrifuged. The supernatant fluid was used as the stock solution. Of this, various amounts were added to the dishes containing the experimental pieces. Onetenth of one ml of stock solution to 10 ml of spring water was found to be optimum. In this concentration, regeneration was most rapid. Higher concentrations than this were lethal. Twenty-two regenerating animals in media containing the optimum amount of pituitary extract showed complete regeneration in 156 to 180 hours. A decrease in total area of about 50% accompanied the regeneration of head pieces. Tail pieces showed a reduction of about 30% in area when regeneration was complete. One-third of the head pieces and one-half of the tail pieces were found to have abnormally large proboseides. The controls, 54 in number, required a minimum of 236 hours for complete regeneration. The reduction in area of the controls was less than 5% at the time of complete regeneration; and no abnormally large proboseides were observed among the control animals.

To delimit further the causative factors of the accelerated regeneration in the presence of whole pituitary extract, 2 series of experiments were carried out. In one of these the experimental pieces were placed in media containing anterior lobe extract only. In the other series, extract of the posterior lobe alone were used. These preparations contained no preservative,† and were employed in dilutions of 0.01 ml to 1 ml per 10 ml of spring water. The animals were transected in the same manner as in the preliminary experiments.

With anterior lobe extract, 166 regenerating pieces were used. Of these, 42 died before showing any regeneration. However, among the 164 control pieces, 42 died so that the deaths among the experimental animals cannot be attributed to the media. The average time for complete regeneration of the planaria in media containing an-

[†] Anterior and posterior extracts supplied by Sharpe & Dohme, Inc., Philadelphia.

terior lobe extract was 450 hours whereas the controls required an average of 504 hours. The average reduction of area for the experimental pieces was 8.5%; for the control pieces, 5%.

In the series of experiments with posterior lobe extract 160 regenerating pieces were tested. The time for complete regeneration for this series averaged 336 hours, an acceleration of 33% over the controls. There was an average reduction in area of 41% as compared with 5% for controls.

Toward the completion of regeneration the control animals divided by fission in 61% of the cases. No fission was observed in the experimental animals in either of the series with posterior and anterior pituitary extracts.

Measurements of respiratory activity after regeneration of 52 hours, in control medium and in concentration of 0.2 ml posterior pituitary extract, per 10 ml spring water gave a Qo₂ for the controls of 4.54. The Qo₂ for the experimental animals was 5.94.

These experiments indicate that regeneration is accelerated in the presence of pituitary extracts, and especially extracts of the posterior lobe. The experimental medium contained 0.17 mg of pituitary material per ml for the tests with posterior pituitary. This together with the fact that the animals decreased in size eliminates feeding as a complicating factor.

Summary. With extract of desiccated whole beef pituitary gland there was a mean increase of 35% in the rate of regeneration of bisected planaria (Euplanaria dorotocephala) with a decrease in total area in head pieces of 50% and a decrease of total area in tail pieces of 30%, when completely regenerated. Posterior lobe extract accelerated regeneration by 33% with a reduction in area of 36% over the controls. The acceleration of regeneration with anterior extract alone was not as marked as with the whole extract or the posterior fraction. There was no reproduction by fission which occurred in 61% of the controls.

11375 P

Evidence for the Local Effect of Mercurial Diuretics.*

R. BEUTNER, J. LANDAY AND A. LIEBERMAN, JR.

From the Department of Pharmacology, Hahnemann Medical College and Hospital

of Philadelphia.

Previous attempts to demonstrate "tissue diuresis" by "mereurial diuretics" failed since the investigated changes of blood colloids or salts were unconvincing. This paper describes certain new observations which may throw light upon the problem.

In previous experiments the inhibition of procaine convulsions by calcium salts was demonstrated.¹ Organic calcium salts were found to be particularly efficient.² Since magnesium salts were found to prevent the anticonvulsive action of calcium salts it seemed that the well known increase of membrane impermeability produced by calcium salts was the chief factor involved in their action.

This property of calcium salts is probably related to their diurctic action. Consequently, we were led to investigate other diurctics, particularly the strong acting mercurial diurctics, such as hydroxy mercuri-methoxy-propyl carbanyl phenoxy acetate, commonly known as "salyrgan". Our expectation was fully justified since salyrgan exhibited an anticonvulsive effect far exceeding that of any calcium salt. The invariably convulsive and oftentimes fatal dose of 200 mg/kg of procaine, intramuscularly in guinea pigs, was rendered entirely harmless by as little as 20 mg/kg of salyrgan simultaneously administered. For comparison, the most effective of all the previously tested calcium salts, calcium benzoate, had to be given in a dose of 50 mg/kg in order to detoxify 200 mg/kg of procaine.

The detoxifying action of salyrgan might be explained as the result of a direct chemical combination with procaine. Various other chemically different convulsant drugs were therefore studied, such as strychnine, picrotoxin, coramin and metrazol. In every instance salyrgan in doses of 10 to 20 mg completely inhibited the action of

[•] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

¹ Beutner, R., and Miley, G. P., PROC. Soc. Exp. BIOL. AND Med., 1938, 38, 279.

² This work was done in collaboration with H. Wastl and A. Jensen (*Ibid.*, 1939, 42, 547). See also the extensive publication of H. Wastl (*Arch. int. Pharmacodyn.*, 1939, 43, fasc. 2) giving quantitative data.

a highly convulsant dose of these drugs. It seems unlikely that salyrgan can combine with any or all of these chemically widely different convulsant drugs. Moreover, various calcium salts such as calcium levulinate, were likewise found to inhibit all of these convulsants. This can hardly be the effect of a direct chemical combination.

The assumption that both salyrgan and calcium salts act primarily on the tissues rendering them more impermeable and *thus* preventing convulsions is further supported by the following findings:

- (1) According to our observations no perceptible inhibition of convulsions occurs in frogs when salyrgan and strychnine, or another convulsant, are injected simultaneously in any lymph sac. Obviously there is a direct access to the nerve cells in this case, membrane permeability being of less importance in the loose tissue of the frogs. If the convulsions were inhibited by direct chemical interaction the inhibition should occur in the frog just as in the mammal injected intramuscularly.
- (2) According to recently published experiments by Spiegel and Spiegel-Adolf' convulsions are accompanied by an increase of the electrical conductivity of the brain tissue, pointing to an increased cell permeability brought about by the convulsing agent. Since calcium salts decrease permeability it seems more than likely that this decrease is the very cause of their anticonvulsive action. The same seems to be true for salyrgan. Since the decrease of permeability is likely to be associated with a dehydration of the tissue, the diuretic action of salyrgan would be explained on the same ground; in other words salyrgan should dehydrate tissues. The water thus eliminated would be ready for excretion through the kidneys.

In contrast to this theory of "tissue-diuresis" by salyrgan, the well known experiments by Govaerts' support the assumption of a renal irritation, favoring glomerular filtration and hindering of tubular reabsorption, as the cause of salyrgan diuresis.

Would it be possible to explain the anticonvulsive action of salyrgan through its renal diuretic effect which possibly leads to an accelerated elimination of the convulsive drug through the kidney? If this were correct salyrgan should also prevent convulsions if injected separately prior to procaine, strychnine, etc. Experiments showed that such an effect does not occur, or, at any rate the inhibition of convulsions is very slight on separate injection.

³ Spiegel, E., and Spiegel-Adolf, M., Proc. Soc. Exp. Biol. AND Med., 1939. 42, S34.

⁴ Govaerts, P., Compt. rend. Soc. de biol. 1928, 99, 647.

The xanthin diuretics were also tested for their possible anticonvulsive action, but were found not to show any such action. Their diuretic effect would, therefore, seem to be of renal origin exclusively. Other heavy metal compounds, however, are likewise anticonvulsive, hence "tissue-diuretic". Detailed data are to be given later.

Conclusion. Mercurial diuretics have a potent anticonvulsive effect. Study of the details of this effect leads to the conclusion that these diuretics increase membrane impermeability, dehydrate tissue and thus work as "tissue diuretics," notwithstanding their well known renal action.

11376 P

Serum Albumin Changes in Hypoproteinemic Dogs Following Administration of Methionine or Phenylalanine.

K. S. Kemmerer and G. P. Heil. (Introduced by Warren M. Cox. Jr.)

From the Mead Johnson Laboratories, Evansville, Indiana.

The observation by Whipple and his coworkers that single amino acids and pure chemical substances increase the production of hemoglobin¹ and serum protein² raises the question of whether observed increases in serum protein after protein feeding are not solely the result of chemical stimulation, or mobilization from body stores of protein, as distinguished from a new synthesis from the ingested protein materials. If so, recorded differences in proteins (when concluded from changes in serum protein levels) would be merely an expression of a difference in amino acid composition and not necessarily express the nutritive value of the proteins for growth or maintenance.

Before such an interpretation can be made, it is essential to have additional data; and especially to determine whether single amino acids under conditions of low dietary protein, as in the procedure of Weech and Goettsch² will effect an increase in serum protein.

¹ Robscheit-Robbins, F. S., and Whipple, G. H., Proc. Soc. Exp. Biol. AND Med., 1939, 41, 361.

² Madden, S. C., Nochren, W. A., Waraich, G. S., and Whipple, G. H., J. Exp. Med., 1939, 69, 721.

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TABLE I.

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Induced by a Low Protein Diet.

-		Plasma alb	umin in g %			Change in plasma
Dog No.	Initial	End 3-week depletion	End 1-week regeneration	End 2-week 1eg.	Supplement 1 g daily during reg.	albumin after 1-wk reg.
31	3.52	2.92	2,50		Phenylalanine	42
32	3.37	2.51	2,13		* * * *	38
21	3.50	2.76	2.51	2.46	,,	25
39	3.41	2.72	2.54	2.48	11	18
28*	2.76	2.03	1.96		17	07
36	3.08	2.48	2,45	2.28	,,	03
29*	2.79	1.28	1.42		,,,	+.14
			Potenc	y value	-0.02 ± 0.051	•
20	2.63	2.16	2.19	2.19	Methionine	+.03
35	3.52	2.66	2.74	2.53	,,,	+.08
2	3.50	2.42	2.51	2.53	,,	+.09
28	3.35	2.61	2.72	2.99	"	+.11
3	3.44	2.27	2.45	2.39	,,,	+.18
2*	3.40	2.27	2.55		7.7	+.28
9*	2.63	1.79	2.12		"	+.33
				cy value	0.307 ± 0.028	,

^{*}Indicates observations made Sept.-Oet., 1939; all others, Dec.-Jan., '39-'40.

Fourteen dogs were used for the study and the above procedure³ followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

The 7 dogs fed 1 g methionine showed an average increase in serum albumin of 0.157 g %. If the Weech and Goettsch figure of 0.15 (to compensate for the fall in albumin that would have been observed during the 4th week if the supplement had not been fed) is added, the potency value of methionine becomes 0.307 ± 0.028 . Phenylalanine resulted in an average drop of -0.17 g %; to which is added 0.15 for a potency value of -0.02 ± 0.051 . Studies in this laboratory would assign a potency value of 0.476^4 to casein. Thus, 1 g methionine under the conditions of the experiment resulted in almost as much increase in serum albumin as did casein, while phenylalanine was not at all effective.

^{*}Purchased from Eastman Kodak Company; amino nitrogen (van Slyke) content of methionine 9.37% or 99.8% of theoretical; of phenylalanine 8.52% or 100.4% of theoretical.

⁴ Mueller, A. J., Kemmeter, K. S., Cox, W. M., Jr., and Barnes, S. T., J. Biol. Chem., in press.

The nanthin diuretics were also tested for their possible anticonvulsive action, but were found not to show any such action. Their diuretic effect would, therefore, seem to be of renal origin exclusively. Other heavy metal compounds, however, are likewise anticonvulsive, hence "tissue-diuretic". Detailed data are to be given later.

Conclusion. Mercurial diuretics have a potent anticonvulsive effect. Study of the details of this effect leads to the conclusion that these diuretics increase membrane impermeability, dehydrate tissue and thus work as "tissue diuretics," notwithstanding their well known renal action.

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28	3.35	2.61	2.72	2.99	11	+.11	
9	3.44	2.27	2,45	2.39	* *	4.18	
2*	3.40	2.27	2,55		9 9	+.28	
9*	2.63	1.79	2.12	••••	* *	+.33	
				ey value	0.307 ± 0.028	,	

^{*}Indicates observations made Sept.-Oct., 1939; all others, Dec.-Jan., '39-'40.

Fourteen dogs were used for the study and the above procedure² followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

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⁴ Mueller, A. J., Kemmerer, K. S., Cox. W. M., Jr., and Barnes, S. T., J. Biol. Chem., in press.

The xanthin diuretics were also tested for their possible anticonvulsive action, but were found not to show any such action. Their diuretic effect would, therefore, seem to be of renal origin exclusively. Other heavy metal compounds, however, are likewise anticonvulsive, hence "tissue-diuretic". Detailed data are to be given later.

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11376 P

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From the Mead Johnson Laboratories. Evansville, Indiana.

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Before such an interpretation can be made, it is essential to have additional data: and especially to determine whether single amino acids under conditions of low dietary protein, as in the procedure of Weech and Goettsch² will effect an increase in serum protein.

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TABLE I.

Effect of Supplements of Methionine and Phenylalanine on Hypoalbuminemia
Induced by a Low Protein Dict.

***************************************		Plasma alb	umin in g %	***************************************		Change in
		End 3-week	End 1-week	End 2-week	Supplement 1 g daily	plasma albumin after
Dog No.	Initial	depletion	regeneration	reg.	during reg.	1-wk reg.
31	3.52	2.92	2,50		Phenylalanine	42
32	3.37	2.51	2.13		-,,	38
21	3.59	2.76	2.51	2.46	7 7	25
39	3.41	2.72	2.54	2.48	* *	18
28*	2.76	2.03	1.96		**	07
36	3.08	2.48	2.45	2.28	"	03
29*	2.79	1.28	1.42		1,	+.14
			Poten	ey value	-0.02 ± 0.051	·
29	2.63	2.16	2.19	2.19	Methionine	+.03
35	3.52	2.66	2.74	2.53	,,	+.08
2	3.50	2.42	2.51	2.53	,,	+.09
28	3.35	2.61	2.72	2.99	,,	+.11
9	3.44	2.27	2.45	2.39	,,	+.18
2*	3.40	2.27	2.55		7.7	+.28
9*	2.63	1.79	2.12		* *	+.33
				ey value	0.307 ± 0.028	, 100

^{*}Indicates observations made Sept.-Oct., 1939; all others, Dec.-Jan., '39-'40.

Fourteen dogs were used for the study and the above procedure² followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

The 7 dogs fed 1 g methionine showed an average increase in serum albumin of 0.157 g %. If the Weech and Goettsch figure of 0.15 (to compensate for the fall in albumin that would have been observed during the 4th week if the supplement had not been fed) is added, the potency value of methionine becomes 0.307 ± 0.028 . Phenylalanine resulted in an average drop of -0.17 g %; to which is added 0.15 for a potency value of -0.02 ± 0.051 . Studies in this laboratory would assign a potency value of 0.476^4 to casein. Thus, 1 g methionine under the conditions of the experiment resulted in almost as much increase in serum albumin as did casein, while phenylalanine was not at all effective.

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11376 P

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TABLE I.

Effect of Supplements of Methionine and Phenylalanine on Hypoalbuminemia
Induced by a Low Protein Diet.

		Plasma alb	umin in g %			Change in plasma
Dog No.	Initial	End 3-week depletion	End 1-week regeneration	End 2-week reg.	Supplement 1 g daily during reg.	albumin after 1-wk reg.
31	3.52	2.92	2,50		Phenylalanine	42
32	3.37	2.51	2.13		***	38
21	3.59	2.76	2.51	2.46	,,,	25
39	3.41	2.72	2.54	2.48	* *	18
28*	2.76	2.03	1.96		1.5	07
36	3.08	2.48	2.45	2.28	"	03
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,			Potenc	er value	-0.02 ± 0.051	
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35	3.52	2.66	2.74	2.53	7.7	+.08
2	3.50	2.42	2.51	2.53	,,	÷.09
28	3.35	2.61	2.72	2.99	9.9	+.11
9	3.44	2.27	2.45	2.39	,,	+.18
2*	3.40	2.27	2.55		,,	+.28
9*	2.63	1.79	2.12		3.5	+.33
				iey value	0.307 ± 0.028	,

^{*}Indicates observations made Sept.-Oct., 1939; all others, Dec.-Jan., '39-'40.

Fourteen dogs were used for the study and the above procedure³ followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

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11376 P

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		Plasma alb	umin in g %			Change in plasma
Dog No.	Initial	End 3-week depletion	End 1-week regeneration	End 2-neek 1eg.	Supplement 1 g daily during reg.	albumin after 1-wk reg.
31	3.52	2.92	2.50		Phenylalanine	-,42
32	3.37	2.51	2.13		,,	38
21	3.59	2.76	2.51	2.46	,,	25
39	3.41	2.72	2.54	2.48	"	18
28*	2.76	2.03	1.96		,,	07
36	3.08	2.48	2.45	2.28	,,	03
29*	2.79	1.28	1.42		,,	+.14
			Poten	ey value	0.02 ± 0.051	-
29	2.63	2.16	2.19	2.19	Methionine	+.03
35	3.52	2.66	2.74	2.53	,,	+.08
2	3.50	2.42	2.51	2.53	"	+.09
28	3.35	2.61	2.72	2.99	71	+.11
Ð	3.44	2.27	2.45	2.39	,,	+.18
2*	3.40	2.27	2.55		,,	+.28
9*	2.63	1.79	2.12		, ,	+.33
			Poten	iey value	0.307 ± 0.028	

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Fourteen dogs were used for the study and the above procedure⁸ followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

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11376 P

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TABLE I.

Effect of Supplements of Methionine and Phenylalanine on Hypoalbuminemia
Induced by a Low Protein Diet.

		Plasma alb	umin in g %			Change in plasma
		End	End	End	Supplement	âlbumin
		3-week	1-week	2-week	1 g daily	after
Dog No.	Initial	depletion	regeneration	reg.	duriug reg.	1-wk reg.
31	3.52	2.92	2.50		Phenylalanine	42
32	3.37	2.51	2.13		• • •	38
21	3.59	2.76	2.51	2.46	> >	25
39	3.41	2.72	2.54	2.48	"	18
28*	2.76	2.03	1.96		* *	07
36	3.08	2.48	2.45	2.28	,,	03
29*	2.79	1.28	1.42		,,,	+.14
			Poteno	r value	-0.02 ± 0.051	
29	2.63	2.16	2.19	2.19	Methionine	+.03
35	3.52	2,66	2.74	2.53	,,	+.08
2	3.50	2.42	2.51	2.53	7 7	+.09
28	3.35	2.61	2.72	2.99	,,	+.11
9	3.44	2.27	2.45	2.39	,,	∔. 18
2*	3.40	2.27	2.55		, ,	+.28
9*	2.63	1.79	2.12		,,	+.33
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^{*}Indicates observations made Sept. Oct., 1939; all others, Dec.-Jan., '39.'40.

Fourteen dogs were used for the study and the above procedure³ followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

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		Plasma alb	umin in g %			Change in plasma
		End	End	End	Supplement	albumin
		3-week	1-week	2-week	1 g daily	after
Dog No.	Initial	depletion	regeneration	reg.	during reg.	1-wk reg.
31	3.52	2.92	2,50	~~~	Phenylalanine	42
32	3,37	2,51	2.13		***	38
21	3.59	2,76	2.51	2.46	, •	25
39	3,41	2.72	2.54	2.48	**	18
28*	2.76	2.03	1.96		"	07
36	3,08	2.48	2.45	2.28	* * *	03
29*	2.79	1.28	1,42		13	+.14
			Potenc	er value	-0.02 ± 0.051	•
29	2.63	2.16	2.19	2.19	Methionine	+.03
35	3.52	2,66	2.74	2.53	• •	+.08
2	3.50	2,42	2.51	2.53	,,	+.09
28	3.35	2.61	2.70	2.99	,,	+.11
9	3.44	2.27	2.45	2.39	, ,	+.18
2*	3,40	2.27	2.55		,,	+.28
9*	2.63	1.79	2.12	,	1 7	+.33
				ey value	820.0 ± 708.0	,

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Fourteen dogs were used for the study and the above procedure³ followed in all details. Total protein, albumin and non-protein nitrogen were run on the plasma from blood taken at the beginning and end of 3-week depletion on the low protein diet, and after one week regeneration. A few observations were made after 2 weeks' regeneration. Supplement consisted of 1 g dl-methionine* or 1 g dl-phenylalanine* added daily to the 53 g per kilo of the basal diet. Table I gives the summarized findings.

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We have had no previous experience with 2 weeks' regeneration, and it is not a part of the Weech and Goettsch procedure. In this instance the change in serum albumin after 2 weeks' regeneration confirms the distinction between methionine and phenylalanine. Other amino acids are being studied in this manner and will be reported later.

11377 P

Role of Particulate Matter in Perfusion of Blood Vessels.*

BENJAMIN W. ZWEIFACH. (Introduced by Robert Chambers.)

From the Laboratory of Cellular Physiology, Department of Biology, Washington

Square College, New York University.

A series of perfusion studies were made in which the capillary vessels of the frog's mesentery were kept under observation through the microscope. Striking differences were found between the circulation obtained with perfusates containing particulate matter and that obtained with similar solutions which were particle-free. Colloidal Ringer perfusates free of particulate matter did not fill all the vessels of the capillary bed, circulating only through the a-v capillaries. In a previous publication, it was pointed out that the a-v capillaries represent direct continuations of the arterioles and serve as bridging channels to the venules. The dye T-1824 (Evans blue, Eastman Kodak Co.) has been used for blood volume studies because of its poor diffusibility.2 When solutions containing Evans blue were used, the restriction of the color to the a-v capillaries stood out in contrast to the true capillaries which remained colorless. The addition of particulate matter, either as a fine suspension of carbon or of washed, rooster red cells, to the Ringer-gelatin or Ringer-acacia perfusates altered the restricted circulation within 30 to 45 seconds by distributing the solution throughout all of the capillaries.

During the early stages of the perfusion with particle-free Ringergelatin solutions, it was observed that the true capillaries were quickly emptied of their contained blood cells. This was peculiar since other observations had shown that the circulation of such perfusates

^{*} This study was made possible by a grant from the Josiah Macy, Jr., Foundation.

¹ Zweifach, B. W., Anat. Rec., 1939, 74, 475.

² Gregerson, M. I., and Gibson, J. G., Am. J. Physiol., 1937, 120, 494.

was sharply restricted to the a-v vessels. By observing the vessels at the commencement of the perfusion, the blood cells in the true capillaries were seen to move simultaneously towards both the arterial and venous ends of the vessels and to be swept into the a-v circulation. This phenomenon appeared to be brought about by a suction effect arising from the rapid streaming of the perfusate past the true capillary orifices in the walls of the a-v channels. The circulation of the particle-free perfusate remained limited to the a-v capillaries throughout the experiment. No change in the character of the circulation was obtained by raising the perfusion pressure from the normal level of 30 mm up to 75 mm Hg. The augmented pressure merely effected a more rapid streaming through the a-v capillaries.

The result obtained when the animal was perfused with Ringergelatin solutions to which a suspension of carbon or avian red cells had been added, was in marked contrast to the above. Under these conditions the circulating fluid not only coursed through the avcapillaries, but streamed into all the capillary side branches. The repeated, alternate use of particle-free and particle-containing solutions in the same preparations emphasized the distinct difference in capillary circulation obtained with the two types of perfusates. The red cells, because of their larger size and extreme plasticity, were more effective in this respect than carbon. The particulate matter appeared to create a series of disturbances at the points of capillary branching,

into the true capillary offshoots.

Ringer-gelatin mixtures, lacking formed elements in suspension, were capable of preventing edema for only 30 to 40 minutes. Ringer-gelatin solutions containing carbon, however, delayed the onset of edema for about 110 minutes. Red cell suspensions were somewhat more efficient, no edema occurring for more than 180 minutes.

thereby disturbing the axial a-v current and deflecting the perfusate

An additional interesting feature of the perfusion with red cell suspensions was the part played by these cells in plugging leaks in the capillary wall. When the capillaries were perfused with artificial solutions for more than 120 minutes, the capillary wall tended to become increasingly porous. Chambers and Zweifach³ have also shown that temporary porous spots appear in the capillary wall and can be increased or decreased by variations in the pH and calcium content of the perfusate. In this stage, a characteristic flattening of red cells against the leaky portions of the wall was observed. This was often followed by portions of the cells being squeezed into tiny openings between the endothelial cells at these points.

³ Chambers, R., and Zweifach, B. W., J. Comp. and Cell. Physiol., 1940, in press.

It is suggested that, as a result of the markedly restricted circulation with particle-free perfusates, abnormal conditions develop which alter the capillary wall and bring about excessive capillary permeability. This would account for the early appearance of edema with such solutions. The widespread distribution of particle-containing perfusates approached a more normal circulation in the capillary bed and was thereby instrumental in pronouncedly delaying the onset of edema.

11378

Attempt to Produce Experimental Cardiospasm in Dogs.*

JACK W. GRONDAHL AND H. F. HANEY.

From the Department of Physiology of the University of Oregon Medical School, Portland, Oregon.

In the clinical condition of cardiospasm food does not pass readily from the esophagus into the stomach although at autopsy the cardia does not exhibit hypertrophy or stenosis.¹ Postmortem studies have shown degeneration of the vagi².³ and loss of ganglion cells⁴-⁰ from the myenteric plexus of the cardia. Failure of the normal receptive relaxation of the cardia in response to the swallowing of food is cited by Hurst¹⁰ as the cause of cardiospasm. Cannon¹¹ demonstrated that this mechanism is abolished in cats following section of the vagi in the neck. By cutting the vagi in the thorax Knight¹² was able to reproduce the X-ray appearance of cardiospasm in anesthetized cats. In the course of a study of the motility of the

^{*} Aided by a grant from the John and Mary R. Markle Foundation.

¹ Sturtevant, M., Arch. Int. Mcd., 1933, 51, 714.

² Heyrovsky, H., Arch. f. klin. Chir., 1913, 100, 703 (Quoted by Lendrum, loc. cit.).

³ Looper, M., and Forestier, J., Arch. d. mal de l'app. digestif., 1921, 11, 306. (See Lendrum, loc. cit.)

⁴ Hurst, A. F., and Rake, G., Quart. J. Med., 1930, 23, 491.

⁵ Cameron, J., Arch. Dis. Childhood, 1927, 2, 358.

⁶ Beattie, W. J. H. M., St. Bartholomen's Hosp. Rep., 1931, 64, 39.

⁷ Mosher and McGregor, Ann. Otol. Rhin. and Laryng., 1928, 37, 12.

⁸ Lendrum, F. C., Arch. Int. Mcd., 1937, 59, 474.

⁹ Hara, H. J., California and Western Medicine, 1929, 30, 390.

¹⁰ Hurst, A. F., J. A. M. A., 1934, 102, 582.

¹¹ Cannon, W. B., Am. J. Physiol., 1904, 19, 436.

¹² Knight, G. C., Brit. J. Surg., 1934, 22, 155.

cardia in dogs Zeller and Burget¹³ performed thoracic vagotomy in nine animals; subsequent studies without anesthesia revealed no loss of tonus or failure of relaxation. They suggest that vagal impulses may be transmitted to the cardia through fibers which travel in the wall of the esophagus.

The present report deals with an attempt to interrupt any such fibers by combining an encircling incision through the outer coats of the esophagus with bilateral thoracic vagotomy.

Since anesthetics interfere with visceral reflexes, a modification of the method outlined by Burget and Zeller14 for recording motility in nonanesthetized dogs was used. Employing this method, the dogs were subjected to preliminary esophagostomy in which the esophagus was brought to the exterior in the midline below the cricoid cartilage. These animals did not regurgitate nor did they lose any considerable amount of food during the act of swallowing. Following recovery the dogs were trained to lie quietly on a table while motility studies were made. Three rubber balloons attached to soft rubber catheters were passed down the esophagus, the lowest being lodged in the cardia, the second in the lower esophagus, and the uppermost a short distance below the esophageal fistula. The 2 lower balloons were connected to sensitive Becker tambours. The uppermost balloon was connected by a T-tube with a rubber bulb and with a mercury manometer. By means of this arrangement stimulation of the esophagus could be effected and recorded. Momentary distension of the uppermost balloon resulted in an almost immediate relaxation of the cardia, which definitely preceded the passage of a peristaltic wave over the middle balloon.

When the dogs were well trained the interruption of nerve pathways in the thorax was attempted. Under nembutal anesthesia and artificial respiration by tracheal catheter, the thorax was entered on the left side, both vagi and the communicating branch were cut about 4 cm above the diaphragm, and the outer coats of the esophagus were cut along its entire circumference at the same level, laying bare a band of white submucosa 1 cm wide. In 5 dogs this procedure was varied by doing the operation in 2 stages, first girdling the esophagus, then reëntering the thorax a few weeks later to cut the vagi.

After operation the animals were observed for difficulty in swallowing, presence or absence of cardiac relaxation, and change of

¹³ Zeller, W. E., and Burget, G. E., Am. J. of Digest. Dis. and Nutration, 1937, 4, 113.

¹⁴ Burget, G. E., and Zeller, W. E., PROC. Soc. EXP. BIOL. AND MED., 1936, 34, 433.

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¹ Sturtevaut, M., Arch. Int. Med., 1933, 51, 714.

² Heyrovsky, H., Arch. f. lin. Chir., 1913, 100, 703 (Quoted by Lendrum, loc. cit.).

³ Loeper, M., and Forestier, J., Acch. d. mal de l'app. digestif., 1921, 11, 306. (See Lendrum, loc. cit.)

⁴ Hurst, A. F., and Rake, G., Quart. J. Med., 1930, 23, 491.

⁵ Cameron, J., Arch. Dis. Childhood, 1927, 2, 358.

⁶ Beattie, W. J. H. M., St. Bartholomew's Hosp. Rep., 1931, 64, 39.

⁷ Mosher and McGregor, Ann. Otol. Rhin. and Laryng., 1928, 37, 12.

⁸ Lendrum, F. C., Arch. Int. Med., 1937, 59, 474.

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¹⁰ Hurst, A. F., J. A. M. A., 1934, 102, 582.

¹¹ Cannon, W. B., Am. J. Physiol., 1904, 19, 436.

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¹⁴ Burget, G. E., and Zeller, W. E., PROC. Soc. EXP. RIOL. AND MED., 1936, 34, 433.

body weight. The dogs subjected to simple girdling of the esophagus alone did not experience difficulty in swallowing, and receptive relaxation of the cardia was present.

All the dogs subjected to the combined operation of girdling of the esophagus and thoracic vagotomy showed marked loss of weight and great difficulty in swallowing. A dog when given a half can of food would gulp it down, immediately begin to strain and extend its neck, and make swallowing movements and soon return practically all of the food as a mucous-coated, sausage shaped mass. The regurgitated food did not change the color of blue litmus paper. repeated attempts the dog might dispose of half a can of food within 10 minutes. In this group of dogs 5 showed complete absence of relaxation of the cardia in all motility records taken postoperatively. Two dogs were too restless following the operations to permit taking satisfactory records, 2 revealed occasional slight relaxation in response to strong stimulation, and one exhibited relaxation which was indistinguishable from that demonstrated in preoperative records. In spite of recorded relaxation these last 3 animals exhibited marked difficulty in swallowing and rapid loss of weight. Of the 12 dogs used 2 died of perforation of the esophagus within 3 days following the thoracic operation. The results are summarized in Table I.

TABLE I.

Operation	No, of dogs studied	No. of dogs showing difficulty of swallowing	No. of dogs in which relaxation could be recorded
Encirclement of esophagus	3	0	5
Combined operation	10*	10	3 f

On postmortem examination the 2 dogs with perforation of the esophagus showed mediastinitis. One died 13 days after operation with hemorrhages of undetermined cause into the stomach and small In this and other dogs sacrificed at intervals of 34 to 84 days after operation, the esophagus was found not to be inflamed, dilated or constricted. In every case one or 2 fingers could be passed easily through the cardiac orifice into the stomach.

The failure to demonstrate uniformly a complete loss of relaxation in 3 of the dogs is not readily explained. It may be that all vagal pathways concerned in the relaxation of the cardia were not severed. It is possible that the motility studies are capable of showing a relaxation of the cardia which is not sufficient in degree to allow the free passage of food into the stomach.

^{*}In 2 of these balloon records could not be obtained. †Two of the 3 exhibited only occasional slight relaxation in response to strong stimulation.

Summary. A combined operation of bilateral vagotomy 4 cm above the diaphragm and girdling of the esophagus at the same level is described as a means of producing in dogs an experimental condition comparable to clinical cardiospasm. In the majority of cases the regurgitation of food can be shown to be accompanied by a failure of receptive relaxation of the cardiac orifice of the stomach. Since neither bilateral vagotomy in the thorax¹⁴ nor girdling the esophagus above the diaphragm is sufficient in itself to produce these results, it may be concluded that some but not all of the fibers responsible for receptive relaxation of the cardia of the dog course downward within the wall of the esophagus.

11379

Failure of Maternal Vitamin A Depletion to Produce Congenital Anomalies in the Young of Rats.

MOTT D. CANNON. (Introduced by W. B. Cannon.)

From the Department of Child Hygiene of the Harrard School of Public Health.

Attention recently has been directed to possible dietary causes of congenital anomalies by Hale's observations^{1, 2, 3} on the occurrence of microphthalmia, hare lip, cleft palate, and the failure of the kidneys to leave their embryonic position in the young of vitamin Adeficient sows. Others who have investigated reproduction in vitamin A-deficient hogs have reported abortion, resorption or the birth of dead fetuses. In some instances prolonged labor was observed, but congenital anomalies in the young were not described.^{4, 5}

Hart, Meade and Guilbert⁶ made no mention of congenital defects in calves born to cows showing night blindness at the time of parturition, nor were anatomical abnormalities recorded by Hart and Miller⁷ among lambs from ewes kept on vitamin A-low rations for nearly a year and night blind at the time of lambing.

¹ Hale, Fred, J. Heredity, 1933, 24, 105.

² Hale, Fred, Am. J. Ophthal., 1935, 18, 1087.

³ Hale, Fred, Texas State J. Med., 1937, 33, 228.

⁴ Hughes, J. S., Aubel, C. E., and Lienhardt, H. F., Kansas Agric. Exp. Sta. Tech. Bull., 1928, 23, 1.

⁵ Hughes, E. H., J. Agric. Res., 1934, 49, 943.

⁶ Hart, G. H., Mead, S. W., and Guilbert, H. R., PROC. Soc. EXP. BIOL. AND MED., 1933, 30, 1230.

⁷ Hart, G. H., and Miller, R. F., J. Agric. Res., 1937, 55, 47.

Sherman and MacLeod, Evans, and Batchelder all found reduced reproductive capacity in vitamin A-deficient rats. was the first to show that lack of vitamin A results in resorption of fetuses, even if abundant vitamin E is provided. This work was confirmed by Mason,12 who showed that vitamin A deprivation also caused appearance of the "placental sign" (red blood cells in the vaginal smear) one to 4 days early, prolonged gestation, and difficult parturition with all or part of the young born dead. Mason makes no mention of congenital anomalies, although he states that all young born to his large series of vitamin A-deficient mothers were routinely examined. Tansley13 and Newton,14 who have confirmed Mason's work, observed no anomalies in the young.

Hale³ states that he has observed 2 cases of congenital blindness in rats from vitamin A-deficient mothers, but mentions that hereditary factors are not ruled out. Browman,15 investigating the reproductive performance of rats receiving adequate vitamin A but which had had a previous history of vitamin A depletion, encountered microphthalmia in one percent of the young born to these mothers. However, 0.7% of the young from the stock colony showed this defect.

In an extensive study of congenitally malformed children, Murphy and Bowes¹⁶ have judged the maternal diet to have been inadequate in 40% of the cases. They do not, however, present control studies on the incidence of inadequate diets among mothers of normal infants.

We have studied the effects of maternal vitamin A deprivation upon the rat fetus, placing particular emphasis upon the question of congenital anomalies.

Two A-low diets were employed:

	A	\mathbf{B}	
Casein (hot alcohol extracted)	24	22	
Sucrose	20	64	
Hydrogenated cottonseed oil (Crisco)	22		
Salt mixture No. 18517	4	4	
Yeast (ether extracted)	10	10	

⁸ Sherman, H. C., and MacLeod, F. L., J. Am. Chem. Soc., 1925, 47, 1658.

⁹ Evans, H. M., J. Biol. Chem., 1928, 77, 651.

¹⁰ Batchelder, E. L., Am. J. Physiol., 1934, 109, 430.

¹¹ Sure, B., J. Agric. Res., 1928, 37, 87.

¹² Mason, K. E., Am. J. Anat., 1935, 57, 303.

¹³ Tansley, K., Biochem. J., 1936, 30, 839.

¹¹ Newton, W. H., J. Physiol., 1938, 92, 32.

¹⁵ Browman, L. G., Am. J. Physiol., 1939, 125, 335.

¹⁶ Murphy, D. P., and Bowes, A. D., Am. J. Obstet. and Gynec., 1939, 37, 460.

¹⁷ McCollum, E. V., Simmonds, N., and Pitz, W., J. Biol. Chem., 1916, 27, 33.

Diet B was supplemented by 0.2 cc of cottonseed oil 3 times weekly. Since performance on these 2 diets does not appear to differ in any important respect, the results will be discussed without reference to the diet used.

Females previously fed Purina dog chow were placed on the described diets at approximately 30 and 60 days of age. They were placed with males at varying periods after the vaginal smears had shown cornified cells continuously.

Of 36 females, mated as proven by the demonstration of sperm in the vagina, 2 failed to conceive. 11 resorbed their fetuses completely,* and 23 carried one or more young to term. These results are tabulated in more detail in Table I.

In our experience, females so depleted as to show xerophthalmia or weight loss generally will not mate. Of the animals mated, the most severely depleted resorbed their fetuses. Those rats which gave birth to young showed gestation periods from 23 to 25 days, with normal labor in some instances, and in others, with labor extending over 12 hours. This confirms the observation of Mason, Tansley, and Newton. It is noteworthy that a similar prolongation of pregnancy with failure of the birth mechanism is induced by the injection of anterior pituitary preparations into pregnant rats. In this latter instance, prolongation of gestation and failure of the

TABLE I.

			ran	121 1.			
Age when placed on A-low diet, days	Days on A-low diet	•	failing to con- ceive		Q which carried 1 or more young to term	Young born or carried to term	% of young alive
60-70	60-70	7	0	1	6	52	76
	100-120	12	0	1	11	81	51
25-30	60-70	7*	2	4	1	3	100
	85-90] †	7	0	2	5	38	52
	100	3	0	3	0		
and 40	A-low diet						
90-110 đ		3	0	0	3	23	100
chow and		11	0	0	11	68	100

^{*}Received 0.25 cc cod liver oil daily after 9-11 day of pregnancy.

[†]Refused to mate and lost weight. Given 120 γ carotene between 60 and 75 days on A-low diet.

^{*} That they had conceived and implanted fetuses was proved by the occurrence of the placental sign.

¹⁸ Teel, H. M., Am. J. Physiol., 1926, 79, 170.

birth mechanism are associated with persistence of abnormal lutein tissue in the ovaries and failure of a new crop of follicles to ripen.

Young born normally or obtained by Caesarian section were minutely examined for abnormalities. The following specific points were checked: ears, eyes, nostrils, lips, roof of mouth, limbs and feet, tail, vertebral column, anus and genital papilla. Except when it was desired to rear the young, the presence of eyeballs was unequivocally established by dissection, and in most instances the viscera, especially the kidneys, were examined. All of the young from A-low mothers appeared anatomically normal except for one which had only the stump of a tail. About half the young were born dead. Resorption sites and embryos in the process of resorption were found even in mothers who bore mature young. The macerated fetuses represented various stages of development, but no anomalies were found where sufficient structure remained to permit of satisfactory examination.

The young reared by A-low mothers (only the less deficient animals would nurse young) were essentially normal in appearance, although somewhat underweight. No evidence of impaired vision was noted. All the young ceased to grow and developed severe xerophthalmia by the time they were 5 weeks old.

Control mothers on dog chow and on the A-low diet plug 40 μ g of carotene 3 times weekly gave birth to living young in every instance after gestation periods of 21 to 23 days. Two of the young from control mothers on dog chow were hydrocephalic.

From these observations, as well as from the work of others already mentioned, it may be seen that, if rat females have sufficient vitamin A to enable them to bear any young, their offspring are anatomically normal. Sheep and cattle appear to behave similarly. Hale emphasized the severity of depletion of his sows, and the possibility should not be overlooked that swine may be able to mate, conceive and carry fetuses when more severely depleted than can rats, sheep, or cattle. If this is true, it is possible that this larger store of vitamin A in rats, necessary for conception, permits the early critical stages of the embryo's development to proceed unaffected.

Summary. 1. Lack of vitamin A failed to induce congenital anomalies in the young of rats. 2. Very severely depleted female rats refuse to mate. In order of decreasing severity of depletion, they will: mate but fail to conceive; conceive but resorb their fetuses; give birth to dead fetuses with difficult and protracted labor after a prolonged gestation; show only prolonged gestation.

11380

N'Dodecanoylsulfanilamide, and Sulfapyridine Plus Vitamin C, in Experimental Tuberculosis in Guinea Pigs.*

M. MAXIM STEINBACH AND CHARLES J. DUCA. (Introduced by C. W. Jungeblut.)

From the Department of Bacteriology, Columbia University College of Physicians and Surgeons, New York.

The striking chemotherapeutic effects obtained recently by the use of sulfanilamide and related compounds have led many workers to study the therapeutic effects of these chemicals on experimental and clinical tuberculosis. The findings reported have been conflicting, and the search for a chemotherapeutic agent which would act efficiently in tuberculosis is still being continued.

As early as 1920, the fatty acids of chaulmoogra oil were suggested for the treatment of tuberculosis (Walker and Sweenev¹), and clinical success in the treatment of skin tuberculosis with hydnocarpic acid has been reported by Rogers² and by Burgess.³ Kolmer and his associates.4 however, showed that chaulmoogric acid does not inhibit the course of experimental tuberculosis in guinea pigs. Recently Crossley, Northey, and Hultquist⁵ synthesized N'dodecanoylsulfanilamide with the thought that a combination of a long chain fatty acid with sulfanilamide might provide a more effective chemotherapeutic agent for tuberculosis than either constituent alone. When this compound was administered to guinea pigs by stomach tube in 100 mg amounts daily for 45 days after infection with H37, Climenko and Schmidt⁶ reported no sign of generalized tuberculosis, as evidenced by the absence of gross involvement of liver or spleen or of general lymphadenitis in these animals. 120 days following infection. Through the courtesy of Dr. D. A. Bryce' we were supplied with generous amounts of N'dodecanoylsulfanilamide for the purpose of studying its action in experi-

^{*} This work was in part supported by a grant from the National Tuberculosis Association.

¹ Walker, E. L., and Sweeney, M. A., J. Inf. Dis., 1920. 26, 238.

² Rogers, L., Brit. Med. J., 1933, 1, 47.

³ Burgess, W., Brit. Med. J., 1935, 2, 835.

⁴ Kolmer, J. A., Davis, L. C., and Jager, R. J. Inf. Dis., 1921, 28, 265.

⁵ Crossley, M. L., Northey, E. H., and Hultquist, M. E., J. Am. Chem. Soc., 1939, 61, 2950.

⁶ Climenko, D. R., and Schmidt, R. L., personal communication.

⁷ Bryce, D. A., Medical Director. Calco Chemical Co.

mental tuberculosis. Throughout the course of this work, tuberculinnegative, male guinea pigs were used.

Experiment I. Eighteen guinea pigs were infected subcutaneously in the region of the groin with 1 mg of human type tubercle bacilli (H37) and divided into 3 groups of 6 animals each as follows:

Group I. Untreated controls.

Group II. Treated with 100 mg N'dodecanoylsulfanilamide in 2% olive oil solution, administered by stomach tube. Treatment started on the day of infection and continued daily for 45 days.

Group III. Treated with 100 mg N'dodccanoylsulfanilamide in 2% olive oil solution, administered by stomach tube. Treatment started 5 days after infection and continued daily for 40 days.

Results. Three control animals died 40 days after infection, 1 animal of Group II died 25 days after infection, and 2 animals of Group III died 40 days after infection. The remaining guinea pigs were sacrificed 60 days after infection. The macroscopic findings, averaged for severity of involvement, are presented in Table I.

TABLE I. Extent of Tuberculosis.

Group	Lungs	Liver	Spleen	Size of spleen	Glands	Summary
III	1.6+	3.0+	2.5+	4.6 × normal	2.1+	2.3+
	1.1+	2.1+	2.1+	3.5 × ''	2.1+	2.0+
	1.2+	2.4+	2.6+	4.8 × ''	2.6+	2.2+

Although the treated animals showed somewhat less disease than the control pigs, there was no evidence of localization and the differences were very slight indeed.

The experiment was, therefore, repeated with a smaller infecting dose. For purpose of comparison, groups of animals treated with sulfapyridine and with sulfapyridine plus vitamin C were included in this series. The latter were added in view of the favorable results obtained previously with vitamin C treatment of experimental tuberculosis.⁸

Experiment II. Thirty-five guinea pigs were infected subcutaneously in the region of the groin with 0.1 mg of H37 and divided into 5 groups of 7 animals each, as follows:

Group I. Untreated controls.

Group II. As in Experiment I.

Group III. As in Experiment I.

Group IV. Treated with 100 mg sulfapyridine in 2% olive oil solution, administered by stomach tube. Treatment started on the day of infection, and continued daily for 45 days.

⁸ Steinbach, M. M., and Klein, S. J., in preparation.

Group V. Treated with 100 mg sulfapyridine in 2% olive oil solution, administered by stomach tube, and 10 mg vitamin C dissolved in normal saline, administered subcutaneously. Treatment started on the day of infection and continued daily for 45 days.

Results. One control animal died 33 days, and 1 died 120 days after infection. One animal in Group III was killed accidentally the day after infection. Two animals in Group IV died 140 days after infection, and 1 guinea pig in Group V died 120 days after infection. Three of the animals in each group were sacrificed 62 days after infection. The findings at this time were the same as obtained in Experiment I. The rest of the animals were sacrificed 140 days after infection. The average autopsy scores, including all the animals of each group, are presented in Table II.

T	'AI	BLE	II.
Extent	of	Tub	erculosis.

Group	Lungs	Liver	Spleen	Size of spleen	Glands	Summary
I III IV V	2.3+ 2.3+ 3.0+ 2.5+ 2.7+	3.0+ 3.0+ 3.4+ 3.5+ 3.4+	3.9+ 2.2+ 3.4+ 2.5+ 3.4+	6.3 × normal 3.0 × '' 5.0 × '' 4.7 × '' 5.2 × ''	3.5+ 2.9+ 2.6+ 2.6+ 3.0+	3.2+ 2.6+ 3.1+ 2.7+ 3.1+

In all groups the infection had spread to every susceptible organ of the guinea pig. The differences which exist in gross appearance and size of spleen between treated and untreated animals cannot be considered significant in view of Corper's report that this effect may be due to the toxic action of the drug. The general impression gathered at the autopsy table was that no group of animals showed significant differences in the extent or degree of tuberculous infection.

Conclusions. (1) Under the conditions of our experiments treatment with N'dodecanoylsulfanilamide shows no inhibitory effect on the course of experimental tuberculosis in guinea pigs infected with the human tubercle bacillus. (2) The administration of sulfapyridine, alone or in conjunction with vitamin C, is ineffective in the treatment of guinea pigs infected with the human tubercle bacillus.

⁹ Corper, H. J., Cohn, M. I., and Bower, C., Am. Rev. Tuberc., 1939, 40, 452.

11381

On the Rôle of Oxalic Acid in Blood Clotting.

R. H. K. Foster. (Introduced by E. Chargaff.)

From the Pharmacology Laboratory, Hoffmann-La Roche, Inc., Nutley, N. J.

Oxalic acid has always been considered an anticoagulant but recently Steinberg and Brown¹ and Schumann² reported that *small* doses administered intravenously hasten coagulation. Oxalic acid is a constituent of normal human blood ranging from 2.75 to 4 mg %²⁻⁶ and somewhat higher in rabbit and beef blood. Steinberg and Brown¹ and Steinberg⁷ stated that oxalic acid and plant extracts containing oxalic acid were found effective in increasing the coagulability of normal blood and also of blood in hemophilia, purpura, obstructive jaundice, vitamin K deficiency, and in prolonged post-surgical bleeding from other causes. (Reference 7 is a popular article by Hannah Lees describing Steinberg's work and so far is the most extensive source of information.) No data were presented by Steinberg concerning the action in vitamin K-deficient animals. Having available vitamin K-free chicks we decided to make a few tests. The effect in normal rabbits was also studied.

Steinberg stated that a coagulant "unit" was the amount of plant extract causing in a 5 lb rabbit 15 minutes after injection a reduction in the clotting time of 50%. He did not give the dosage of oxalic acid for rabbits, but mentioned the human dosage to be 3 mg. A corresponding dosage in proportion to body weight, with a considerable range on either side, was used in rabbits and chicks.

Coagulation times were determined by a modified Howell method. Blood was drawn into an oiled syringe and transferred to a vaccine tube which was immediately stoppered with a paraffined cork and placed in a water bath. The tube was tilted every half minute until clotting occurred. From chicks 0.2 cc of blood was drawn from the brachial wing vein and from rabbits 1.0 cc by heart puncture.

It is well known that after repeated veni- or heart-punctures increased coagulability may sometimes be observed. Controls were run to rule out this factor and the data are listed in Tables I and II.

¹ Steinberg, A., and Brown, W. R., Am. J. Physiol., 1939, 126, 638.

² Schumann, E. A., Am. J. Obst. and Gyn., 1939, 38, 1002.

³ Magerl, J. F., and Rittmann, R., Klin. Wochschr., 1938, 17, 1078.

⁴ Merz, K. W., and Maugeri, S., Z. Physiol. Chem., 1931, 201, 31.

⁵ Suzuki, S., Jap. J. Med. Sci., II Biochem., 1934, 2, 291.

⁶ Kamiya, S., Jap. J. Mcd. Sci. II Biochem., 1937, 3, 163.

⁷ Lees, Hannah, Collier's, 1939, Sept. 23, p. 48.

TABLE I. Clotting Tests on Rabbits.

		Clott	ing times
Animal No.	Dosc per kg	Before	15 min after
303 306 317 318 319	Control—no injection	2+ 3 3 2+ 3	3+ 3+ 2+ 3 2+
304 307	Saline—1/2 ce	3 2+	3+ 4+
325 326 328 322 261 280 314 270 315 304 309	Oxalie acid— 10 γ '' '' 20 '' '' '' 40 '' '' '' 80 '' '' '' 125 '' '' '' 175 '' '' '' 450 '' '' '' 600 ''	+ 23324323333	2+ 2+ 4+ 2+ 2+ 3+ 2+ 3 2+ 3 5 hours

⁺ indicates half minute.

TABLE II. Clotting Tests on Chicks.

Group No.	Condition of chicks	Dose γ/chick	N	Clotting times—min
1-3	Normal	Saline 0.02 cc	9	2+, 2+, 3+, 3+, 5+ 4+, 4+, 7, 7
2-4	,,	Oxalic acid 2 ₇	9	1+, 2, 2+, 2+, 4, 5+
5-19-22	K-free	Control	12	88, 106, 10 > 120
6	"	Venipuncture	4	All >120
7-20-23	"	Saline 0.05 cc	13	$31\ 12 > 120$
8	"	Oxalic acid 1 -	3	All >30
9-10-11	"	Oxalic acid 1 y	, 3	50, 92, 105, >30, >180
12	"	,, ,, ,, ,	, 3	All >30
24	"	,, ,, 3,5 ,		All >30
13-14-15-21	1)	" " 5	-	18, 25, 99, 8 >120
16-17	,,	" " ₁₀ "		41, 46, 2 > 120
18	"	$,,,,\frac{10}{25},$		50, 73

A dose of oxalic acid of 40-50 γ /kg corresponds to a 3 mg human dose. As seen in Table I this dose was without effect in normal rabbits and the only significant effect obtained was with a dose of 600 γ /kg, which caused a great prolongation of the clotting time. Data on 8 other rabbits are omitted from the table to save space. The results were no different. Since the normal value of oxalic acid in rabbits is 6-9 mg $\%^4$ a 600 γ dose would correspond to an increase in the oxalic acid content of the blood of about 15%. The dose

recommended (40-50 γ /kg) as eausing a shortening of the clotting time amounts to searcely a 1% increase in the oxalic acid concentration. It hardly seems logical that so small an increase could affect the clotting time in either direction unless there was no free oxalate in the blood to start with. But whether the normal oxalate is combined or free, or whatever may be its function in blood, these data do not support the view that it possesses any coagulant effect whatsoever in normal blood

Table II gives tests on normal and vitamin K-free chicks (Almquist' diet). The chicks were 2 weeks old at the time of testing and most of them showed the characteristic hemorrhages caused by prothrombin deficiency resulting from an inadequate vitamin K supply. Doses of oxalic acid varying from 1γ to 25γ per chick were used and in no case was the clotting time reduced to normal. A dose of 3.5 γ per chick corresponds to the human dose given by Steinberg. Two chicks showed clotting times of less than 30 minutes, but this is not significant since controls occasionally showed similar results. Although one of the saline controls clotted at 31 minutes, the majority showed no clotting within 2 or 3 hours. Administration of vitamin K returned the clotting time to normal (not included in table). In normal chicks a dose of 2γ per chick had no effect. The chicks used in the entire series ranged in weight from 50 to 145 g with an average of 98 g for 83 chicks.

Rabbit blood made deficient in coagulability by administering near threshold doses of heparin intravenously was unaffected by small intravenous doses of oxalit acid. With the doses of heparin used the clotting time returned to normal in about one hour. Heparin is generally considered as antiprothrombin and the heparinized animal is therefore comparable to the vitamin K-deficient chiek, prothrombin being absent (or greatly diminished) in the latter and immobilized in the former. Only a few tests were run, and while the data were limited, there was no indication of any effect in the doses used nor evidence warranting more extended trials.

The literature describes oxalic acid as being a normal constituent of blood and other body tissues. The concentration changes in various conditions but there is no evident parallelism to changes in coagulation. During narcosis it increases in bile and in the urine.^{0, 10} This increase is attributed to anoxemia and Kamiya²¹ and others

⁸ Almquist, H. J., J. Biol. Chem., 1936, 114, 241.

⁹ Borgstroem, S., Skand. arch. Physiol., 1937, 77, 16.

¹⁰ Borgstroem, S., Skand. arch. Physiol., 1938, 79, 1.

¹¹ Kamiya, S., Jap. J. Med. Sci. II, Biochem., 1937, 3, 301.

showed that anoxemia in rabbits increased the oxalic acid content of blood by as much as 60%. Kamiya6 and Marcolongo12 showed that it rose in high blood pressure, uremia, ruberculosis, syphilis, beri beri, neuralgia, rheumatism, cirrhosis, and in acute and chronic nephritis. Melocchi¹³ observed a rise of oxalic acid in the blood during intestinal fermentation of carbohydrates. Olson also made the comment in a letter quoted by Schumann' that on the basis of the amount of oxalic acid normally in the blood, it would not seem logical that a small increase could have any effect on the clotting time. The experimental evidence bears out Olson's view.

Conclusions. Oxalic acid injected into animals over a wide range of dosage was found to have no effect on coagulation until a sufficiently high dose level was reached, at which point clotting was delayed. Included in this study were suitable controls, normal and heparinized rabbits and normal and vitamin K-deficient chicks.

11382 P

Direct Observations on the Circulation of Blood in Transilluminated Mammalian Spleens.*

DAVID W. MACKENZIE, JR., ALLEN O. WHIPPLE AND MARGARET P. WINTERSTEINER. (Introduced by A. M. Pappenheimer.)

From the Department of Surgery, Columbia University College of Physicians and Surgeons, New York, N. Y.

The spleens of living mice, rats, rabbits, guinea pigs and cats were transilluminated and observed at several magnifications, as high as 600 X (water immersion). Each type of spleen was completely delivered through a long paracostal incision and placed in a suitable celluloid chamber, on a light table, above the abdominal wall. this means, respiratory motions were eliminated in some, and greatly reduced in all specimens. The spleen, thus supported, was totally immersed in rapidly flowing Ringer-Locke solution at 37°-38°C. Anesthesia hypodermoclyses of sodium iso-amyl ethyl barbiturate

¹² Marcolongo, F., Clin. med. ital., 1934, 65, 1068. 13 Melocchi, W., Giorn. clin. med., 1934, 15, 1669.

^{*} The cooperation of the Department of Pathology, greatly facilitated this work.

¹ Knisely, M. H., Anat. Rec., 1938, 71, 503.

recommended (40-50 γ/kg) as causing a shortening of the elotting time amounts to scarcely a 1% increase in the oxalic aeid concentration. It hardly seems logical that so small an increase could affect the clotting time in either direction unless there was no free oxalate in the blood to start with. But whether the normal oxalate is combined or free, or whatever may be its function in blood, these data do not support the view that it possesses any coagulant effect whatsoever in normal blood.

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scale, the cyclical blood flow observed by Knisely2,3,4 in the venous sinuses.

The venous sinuses and intralobular veins are passive recipients of blood. Their walls reveal innumerable stigmata, offering little or no obstruction to the influx of erythrocytes, and permitting the passage of lymphocytes and larger white cells with varying degree of distortion. In the absence of extrinsic interference with venous drainage, the sinuses swiftly transmit to the larger venous tributaries the blood which enters them from the pulp spaces. The only vascular sphincteric action that we have observed in the spleen pulp is exhibited by the arterioles and arterial capillaries, which are intermittently constricted, either individually or in groups.

Agonal vascular disintegration, as described by Knisely,4 has not been apparent in our preparations. Because of extreme capsular contraction, in the absence of positive arterial blood-pressure, the pulp of the dying spleen pales to a degree that is never otherwise attained. Having seen intracellular erythrocytes in living, normal splenic pulp, we are not satisfied with Knisely's assumption that phagocytosis of red cells is, in this situation, a purely agonal phenomenon. In effect, the spleen pulp acts as a filter, separating red cells from plasma, and removing foreign particulate matter, including certain erythrocytes, from the circulating blood. We have watched the appearance of India ink particles, inside pulp phagocytes, less than 20 seconds following their peripheral intravenous injection.

From the standpoint of reservoir function, the reaction which might be termed the 'emergency response' of the spleen, as classically demonstrated in the gross by Barcroft^{5, 6} and his colleagues. was consistently reproduced in our experiments by such factors as electrical stimulation of the pedicle nerves, adrenalin, exercise, anoxemia, hemorrhage and temperature changes. Microscopically, the response consisted of pulp compression and arterial constriction, leading to obliteration of the extravascular spaces and consequent mobilization of a quantity of relatively concentrated red cells.

Because of the pitfalls inherent in the technic of living tissue transillumination, our conclusions are necessarily tentative. We are reasonably convinced, however, that the circulatory systems of the spleens we have examined are 'open'; that, in other words, they lack

² Knisely, M. H., Proc. Soc. Exp. Biol. and Med., 1934, 32, 212.

³ Knisely, M. H., Anat. Rec., 1936, 65, 23

⁴ Knisely, M. H., Anat. Rec., 1936, 65, 131.

⁵ Barcroft, J., Lancet, 1925, 1, 319.

⁶ Barcroft, J., Lancet, 1926, 1, 544.

(sodium amytal, Lilly) provided adequate narcosis without disturbing the animal's position. Methods were devised for variously stimulating the spleen during the period of observation. The albino mouse was studied in greatest detail. It was noted, however, that the circulatory mechanisms of all species investigated presented fundamental similarities, which seemed to justify the following generalizations.

The afferent capillaries of the pulp communicate with naked pulp spaces at the point where their terminations are marked by ampullary dilatations and diminished mural refractility. No preformed, intactly lined connections between the arterial and venous systems in the spleen have been as yet conclusively demonstrated in our preparations. The break in vascular continuity is obvious in a relaxed or distended spleen, where the walls of the pulp channels are seen to be composed of spherical, oval or polyhedral cells and vague, linear shadows, suggestive of reticulum fibers; but, when the spleen is contracted, it is difficult to detect, because the residual channels, surrounded by compressed pulp cells, then assume the appearance of completely walled vessels, connecting arterial capillaries with venous sinuses and intralobular veins.

The capacity of the pulp is a function of capsular contraction, which takes place rhythmically as well as in response to specific stimulation. The behavior of the individual pulp spaces is additionally governed by arterial and venous blood-pressure variations, relationships to neighboring channels, the obstructing action of migrating leucocytes, derived either from the pulp cords or from associated arterial capillaries, and by swelling or shrinkage of the reticular stroma.

The pulp space is the most variable structure in the transilluminated spleen. It may convey, at different times, plasma almost devoid of red cells, rapidly flowing blood of normal cellular content, or slowly oozing, highly concentrated red cells. When the narrower, efferent end of the pulp channel is blocked by transient leucocytes, or by some other factor, blood accumulates within it. If, at this time, the adjacent pulp cells happen to be compressed, the channel becomes distended, and the blood within it acquires such concentration that the contours of individual erythrocytes are scarcely visible. In the absence of pulp compression and concomitant arterial constriction, the column of blood is obstructed momentarily, but rapidly alters its course and penetrates alternative passages to the nearest venous tributary. In our experience, this intermittency of circulation in the pulp most closely resembles, on a much smaller

scale, the cyclical blood flow observed by Knisely2,3,4 in the venous sinuses.

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vertical position. Current was supplied to the arm baths at 11,160 cycles/second by the measuring instrument.³ About 90% of the resistance measured by this method is due to the unimmersed segment.⁴ Measurements over a control period of 2 hours were reproducible within $\pm 2\%$ and were made one minute after immersion.

The test subject excreted 150 cc of urine during the injection period (1 hour). Although the volume of retained saline (about 850 cc) was less than 2% of the total body weight (50 kg), a decrease of 10% in the measured resistance was observed. Confirming tests on a number of additional subjects yielded changes of the same order.

This surprisingly large decrease in body resistance demanded an explanation and the following working hypothesis was adopted. It is known that, when isotonic saline is injected intravenously, it does not distribute itself uniformly throughout the tissues but, after leaving the vascular system, accumulates in the extracellular or interstitial spaces.5 Since these spaces are estimated to constitute only 20-30% of the total tissue volume, a given volume of saline entering an extremity such as the arm, either fills in or increases the cross-section of the interstitial spaces by 3-5 times the amount that it would if it were also taken up intracellularly. The interstitial spaces in the arm lie in parallel relation to the muscle fibers. Current flowing along the arm splits up into parallel paths, part flowing along the inside of the muscle fibers and the rest along the interstitial spaces outside the latter. In the chest segment the current splits to flow in parallel paths through the chest muscles on the one hand and through the lung on the other. The reduction in resistance of the interstitial branches of these parallel circuits is the cause of the comparatively large resistance drop.

The distribution of extracellular isotonic saline following intravenous injection is variously reported in the literature. Skelton, working with cats, found that none of the saline reached the muscles but was absorbed mainly by the skin. Hastings and Eichelberger, on the contrary, working with dogs, traced considerable saline into the extracellular spaces of the muscles. The subject has been reviewed by Adolph. From the electrical standpoint, whether the saline accumulates between the muscle fibers or in the subcutaneous

⁴ Horton, J. W., Van Rayenswaay, A. C., Hertz, S., and Thorne, G., Endocrin., 1936, 20, 72.

⁵ Peters, J. P., Body Water, C. C. Thomas, Baltimore, 1935.

⁶ Skelton, H. P., PROC. Soc. EXP. BIOL. AND MED., 1925-6, 23, 499.

⁷ Hastings, A. B., and Eichelberger, L., J. Biol. Chem., 1937, 117, 73.

⁸ Adolph, E. F., Physiol. Rev., 1933, 13, 336.

the type of connection which commonly links arterial and venous capillary networks; that the pulp space—not the venous sinus—is the primary physiological unit of the splenic vascular mechanism; and that contraction of the capsule and trabeculae may convert the structurally 'open' circulation of a relaxed or distended spleen into a functionally 'closed' eirenit.

11383

Electrical Method for Studying Water Metabolism and Translocation in Body Segments.

A. BARNETT. (Introduced by M. M. Harris.)

From the Department of Psychiatry, N. Y. State Psychiatric Institute and Hospital, New York City.

Although the electrical resistance of a munimified arm would, obviously, be very much greater than that of a fresh cadaver, no serious attempt has ever been made to measure the water content of a body segment in terms of its electrical resistance. One of the reasons for this would appear to have been the lack, until comparatively recently, of technics which would permit resistance measurements to be made. Using alternating current, it has now become possible to measure, with a fair degree of accuracy, not only the resistance of various parts of the arm-to-arm segment in man (the upper arm, the chest segment alone or the arm and chest segments together) but also changes in their dielectric properties.^{1, 2, 3}

Hydration. In order to test out the possibility of measuring body water changes electrically, a liter of isotonic saline solution was injected intravenously into a normal individual weighing 50 kg who had been deprived of fluids for 3 hours previously and the electrical resistance of the arm-to-arm segment was measured before and 30 minutes after the injection. Resistance measurements were made by the immersion method at room temperature. The subject stood before a table 32 inches high and immersed the forearms in 9 liters of normal saline (11 cm deep) contained in a pair of arm baths supported on the table so that (1) the elbows rested on the arm-bath bottoms and (2) the upper arms were in a substantially

¹ Horton, J. W., and Van Ravenswaay, A. C., J. Frank. Inst., 1935, 220, 557.

² Barnett, A., and Bagno, S., Proc. Soc. Exp. Biol. and Med., 1937, 36, 543.

³ Barnett, A., West. J. Surg., 1937, 45, 380.

TABLE I. Effect of Intravenous Injection of One Liter of Isotonic Salt Solution on the A.C. Resistance of the Arm-to-arm Segment and on the Q-factor of a 10 cm Arm Segment.

				;	Resistar	Resistance in ohms by the immersion method	, by the shod	Q-fn	tetor of a l trm segmen	.0 cm nt
Subj.	Sex	γgο	Wt in kg	Saime retained ee	Before in jection	ore After 9 tion injection differ	% difference	Before After % injection differen	After injection	% difference
1.6	0	15	40.3	006	275	뚦	10.9	990	.058	12.1
17 TO	+ 0	26	46.9	096	235	202	12.8	100	.077	£.72
IN	+ 0	(C)	44.0	700	306	275	10.1	070	.058	17.2
M. B.	+0	10 10	53.6	975	315	282	10.5	.078	₹90.	17.9
2	+0		63,4	800	210	185	13.3	.078	890.	12.8
	+0	19	64.1	750	9.00 70.00	200	11.1	101.	080	11.9
A.B.	+ 0	95	43,6	440	345	314	0.0	.081	.063	61 61 61
<u>.</u>	+0	£3	50.7	850	065	202	9.6	.102	080	21.6
ĭ.D.	+ *(<u></u>	6.49	006	252	200	5.6	.123	.102	17.1
L.B.	o*o	30	50.9	800	534	194	17.1	.102	.087	14.7
						Mean	ın 11.4%		M	2an 17.5%

tissues, the current path still remains a parallel one and the infiltrated saline shunts the intracellular branch of the circuit.

Since the cell walls in the arm are known to be dielectrics having capacitative properties, a change in arm resistance should modify the Q-factor which is the ratio between the reactances, due to these capacitances, and the resistance. Q-factor measurements were, therefore, made of a 10 cm length of the deep tissues of the right upper arm by the 4-electrode technic^{1, 2, 5} before and after the injection of a liter of saline.

Results are given in Table I for 10 subjects on whom measurements of both resistance and Q-factor were made. Nine of these subjects were mental patients with negative physical findings.

The resistance changes vary from 9 to 17% with a mean of 11.4% and the Q-factors from 12 to 27%—mean 17.5%. Normal subject A.B. is remarkable for the large changes in the electrical values despite the retention of only 440 cc of saline. Subject C.P. was given an injection of 2 liters of saline over a period of 2 hours. 1250 cc were retained. The resistance change was 60 ohms and the Q-factor decreased 0.028 (27%). Subjects C.P. and M.B. were given injections of one liter of Ringer's solution instead of isotonic saline. No significant difference in the order of the electrical changes was observed.

Resistance measurements were made on 3 subjects 3½ hours and 24 hours after the end of the saline injection. The resistances after the 3½-hour interval were still decreased but had moved back towards the preinjection level roughly in proportion to the volume of urine excreted which took place at the rate of approximately 100 cc per hour. At the end of 24 hours, the resistances had returned to the preinjection levels.

Blood pressure measurements made before, after and during saline injections showed variations of the order of ± 10 mm which may be considered as not significant.

Dehydration. As a test of the reversibility of resistance measurements, a female subject weighing 63 kg and normally excreting 1500-1700 cc of urine daily, was put on a restricted fluid intake of 500 cc daily and 50 cc of 50% glucose was given intravenously twice daily for 3 days. Her body resistance was 200 ohms at the beginning of the experiment. On the fourth day, there had been a total weight loss of 2 kg, the urine output had descended to 600 cc for the previous 24-hour period and the body resistance had risen 45 ohms, from 200 to 245 ohms.

Clinical Applications. (1) A female patient 16 years of age and

factor decrease following infusion of isotonic saline. This is to be expected in a parallel type of circuit. In a circuit of the series type, a decrease in resistance would result in an increased Q. The studies here presented are of a preliminary nature and do not permit a judgment as to the contributory rôle of the various anatomical elements lying along the current path to the total result. A rough analysis of the probable relative effects of the extracellular and intracellular phases has been presented for the particular case where isotonic saline is injected intravenously. It is possible that vascular factors also intervene. Studies along these lines are now in progress.

I am indebted to A. N. Mayers for assistance during part of this investigation.

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Action and Toxicity of Vitamin B. Hydrochloride.

C. G. WEIGAND. CHARLES R. ECKLER AND K. K. CHEN. From the Lilly Research Laboratories, Eli Lilly and Company, Indianapolis.

In view of the increasing interest in vitamin B_0^{1-1} as a nutritional accessory, the present investigation was undertaken in order to determine the degree of potency and limits of toxicity. All experiments were carried out with crystalline vitamin B_0 hydrochloride, m.p. 212°C (corrected) with effervescence.

General Properties. Vitamin B_n · HCl is easily soluble in water. Its aqueous solutions are acid in reaction. A 1% solution has a pH of 2.44. In vitro a quantity of 4 mg caused hemolysis of sheep's washed erythrocytes, but if it was previously neutralized, no laking took place. Obviously the hemolytic effect was due to the acidity. When a 1% solution of B_n · HCl in the amount of 0.5 cc was injected both subcutaneously and intramuscularly into 3 rabbits, practically no irritation occurred, but when 0.1 cc of the same

¹ György, P., Nature, 1934, 133, 498; J. Am. Chem. Soc., 1938, 60, 983.

² Fouts, P. J., Helmer, O. M., and Lepkovsky, S., Proc. Soc. Exp. Biol. and Med., 1939, 40, 4; Am. J. Med. Sc., 1940, 199, 163.

³ Spies, T. C., Beau, W. B., and Ashe, W. F., J. A. M. A., 1939, 112, 2414.

⁴ Kark, R., Lozuer, E. L., and Meiklejohn, A. P., Proc. Soc. Exp. Biol. and Med., 1940, 43, 97.

weighing 82 lb diagnosed as an hysteric had lost weight progressively as a result of prolonged vomiting. The vomiting episodes lasted for periods of several days during which time the patient regurgitated all food and liquid immediately after ingestion by inserting her fingers into her mouth to induce the vomiting reflex. The body resistance measured by the immersion method after 2 days of continual vomiting was the highest ever observed in a human subject—445 ohms. Suddenly the vomiting eeased and the patient ingested large quantities of food and liquid over a period of 48 hours. There was a gain in weight of 1½ kg. At this point the body resistance was found to be 405 ohms. An injection of one liter of isotonic saline was then given over a period of one hour intravenously. 745 ce were retained. The resistance fell from 405 to 334 ohms, a change of 71 ohms.

- (2) Resistance measurements were made in 2 cases of involutional melancholia (females) under treatment with testosterone propionate which is now widely used for menopausal disturbances.⁹ 25 mg were given intramuseularly thrice weekly. Zuckerman and Bourne¹⁰ have shown that, in primates, injections of testosterone propionate cause water retention. Recently Kenyon¹¹ and his coworkers have reported that this substance also produces water retention in man (normals). In both cases under treatment a progressive drop totaling about 10% in body resistance was observed in the course of a month. The absolute changes were 290 to 260 ohms and 285 to 251 ohms. Spontaneous resistance changes of this magnitude are not observed in conditions of this kind.
- (3) Transverse resistance measurements were made on a female exhibiting a cyclic oedema of the ankles coincident with each menstrual period. The eircumference at the ankle level increased by 4 cm during periods. Concentric electrodes^{12, 13} were mounted on the leg by means of rubber bands at a level 3 inches above the ankle so that the current passed diametrically from the internal to the external lateral sides and the resistance of the deep tissues between electrodes was measured at, and 2 weeks after, a period. The measured resistance increased from 118 during oedematous infiltration to 212 ohms, when the oedema had been resorbed.

Remarks. It is important to note that both the resistance and Q-

⁹ Kurzrock, L., Birnberg C. H., and Livingston, S., Endocrin., 1939, 24, 347

 ¹⁰ Zuckerman, S., Palmer, A., and Bourne, G., Nature, 1939, 143, 521.
 11 Kenyon, A. T., Knowlton, K., Sandiford, I., Koch, F. C., and Litwin, G., Endocrin, 1940, 26, 26.

¹² Bainett, A, J. Physiol, 1938, 93, 349.

¹³ Barnett, A., West. J. Surg., 1937, 45, 322.

by subcutaneous and oral administration has been estimated by Unna and Antopol.

Repeated Administration. Five mice were injected by the tail vein with daily doses of 100 mg per kg except Saturdays and Sundays for 2 weeks. All of them gained weight during the course of medication. Upon sacrifice, no pathological changes were detected.

In Men. Twelve young male adults consented to take $B_e \cdot HCl$ by different routes as shown in Table II. The drug was dispensed in capsules for oral use, but made into 2.5% solution for injection purposes. Briefly, it may be stated that no ill effects were observed when $B_e \cdot HCl$ was given either by mouth or by intravenous injection in the dosage of 100 to 200 mg. Pain uniformly occurred when the drug was injected intramuscularly, perhaps due to its

Action of Vitamin B₆. HCl in Men by Various Routes of Administration.

			Dose, mg		
Subject No.	Age	Oral	Intra- muscular	Intra- venous	
1	22	100	50	100	None "Sore" at site of injection for 11/2 hours None
2	42	100			"
3	35	100			77
4	40	100			27
5	24	100	50	100	"Aching" for 15 min None
6	33		50	100	"Burning" for 15 min None
7	28		50	100	"Sore" for 2 hrs None
8	30		50	100	"Sore" for 15 min None
9	28		50	100	"Aching" for 2 hrs None
10	21			200	"
11	29			200	71
12	21			200	"

e Unna, K., and Antopol, W., Proc. Soc. Exp. Biol. AND Med., 1940, 43, 116.

solution was injected intracutaneously, congestion was evident for more than 4 days in 2 out of 3 animals.

Potency. In addition to physical and chemical characterization. crystalline vitamin B₀ may be assayed biologically. For this purpose, young rats of 21 to 23 days of age were fed a ration composed of sucrose 67%, vitamin-free casein 20%, McCollum's salt mixture No. 185 4%, Crisco 3%, liver filtrate free from Be 2%. cod liver oil 2%, and agar 2%. In addition, each animal received daily doses of 20 y each of thiamin chloride and riboflavin. Towards the end of 6 to 8 weeks, these rats developed typical signs of B₆avitaminosis-acrodynia and inhibition of growth. They were then treated individually with single doses of various size by mouth, and examined daily for 2 weeks. In a group of 60 rats, it was found that a dose of 40 to 60 7 cured acrodynia in an overwhelming majority of the rats within 14 days. Meanwhile, there was a decisive increase in their body weights. These results are comparable to those of Reedman, Sampson, and Unna.5 For testing the potency of a new lot of Bo HCl, it has been the practice of this laboratory to determine the median curative dose for acrodynia (CD₅₀), and compare it with the CD₅₀, determined simultaneously, of the purest lot which is being preserved as a standard.

Acute Toxicity. By intravenous injection, the median lethal dose (LD₅₀) of B₆. HCl in mice was found to be 545.3 ± 42.9 mg per kg, and that in rats 657.5 ± 18.3 mg per kg. The data are shown in Table I. Tonic and then elonic convulsions occurred, and death followed rapidly. Animals either completely recovered or succumbed within 5 minutes after injection. No after effects were noted in the surviving rats. The acute toxicity of B₆. HCl in rats

TABLE I.

Toxicity of Vitamin B6 . HCl in Mice and Rats by Intravenous Injection.

Animal	Conc. of solution,	Dose, mg per kg	No. died No. used	LD ₅₀ ± Standard error, mg per kg
Mice		300	0/5	545.3 ± 42.9
A11100		400	1/5	
		500	1/5	
		600	3/5	
		700	3/3	
70.45	5	500	0/3	657.5 ± 18.3
Rats	J	600	1/5	
		650	4/10	
		700	4/5	

⁵ Reedman, E. J., Sampson, W. L., and Unna, K., Proc. Soc. Exp. Biol. AND Med., 1940, 43, 112.

changes. 5. No ill effects occur in men when $B_{\sigma} \cdot HCl$ is administered orally or intravenously in the dosage of 100 to 200 mg. Pain occurs when the drug is injected intranuscularly. 6. In the concentration of 1:8000, $B_{\sigma} \cdot HCl$ inhibits isolated rabbit's small intestines, but contracts the isolated guinea pig's uterus.

11385

Effects of Subcutaneous Injection of Estrogen upon Skeleton in Immature Mice.

CHARLES J. SUTRO. (Introduced by H. L. Jaffe.)

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It is known that the subcutaneous administration of estrogen produces osteosclerosis in immature mice. Hitherto, however, detailed roentgenographic and histologic studies explaining the mode of development of the osteosclerosis have been lacking. This paper reports such an investigation.

The study was conducted upon 66 immature mice, some of the C₃H strain and some from stock colony of the Rockland Farms. The experimental subjects received subcutaneous injections of estradiol benzoate in sesame oil* each week for a certain number of weeks. The control animals received injections of sesame oil for corresponding periods of time.

At the expiration of the total period allotted for the experiment, the animals were roentgenographed and autopsied. The skeletal tissues [femur, tibia, humerus, vertebral column, calvarium, ribs, pelvis, foot and jaw (including incisors)] were fixed in Helly's fluid, decalcified in 5% nitric acid, embedded in paraffin and stained with hematoxylin and eosin. The soft tissues were likewise prepared for histologic examination.

Table I summarizes the organization of the experiments. The control mice received weekly injections of sesame oil.

Roentgenographic and Gross Pathologic Findings: Roentgenographic examination reveals that in immature mice osteosclerosis, caused by the formation of new bone in the medullary cavity, especially in the lower end of the femur and upper end of the tibia,

¹ Gardner, W. U., and Pfeiffer, C. A., PRCC. Soc. Exp. Biol. And Med., 1938, 37, 678; ibid., 1938, 38, 599.

^{*} Progynon-B was generously supplied by Schering Corporation.

acidity. In 3 out of 6 cases, it lasted as long as $1\frac{1}{2}$ to 2 hours. No other toxic manifestations were noted.

Other Effects. Casual observations were made on cats' blood pressure and respiration. Doses of 100 mg did not alter the pulse nor the respiratory rate; nor did they change the height of carotid pressure. Appropriate concentrations (1:8000) of B₆: HCl caused brief inhibition of isolated rabbit's small intestines with prompt recovery, and contraction of the isolated guinea pig's uterus. The response here was not due to a low pH, because controls with the same acidity did not reproduce these results.

Summary. 1. Vitamin B_a: HCl is acid in reaction which may be responsible for certain irritating effects in body tissues. 2. A method of bioassay has been described based upon the curing of rats' acrodynia. 3. The acute toxicity of B_a: HCl in mice and rats by intravenous injection has been determined. 4. Mice can tolerate repeated doses of 100 mg per kg, given intravenously, without pathological



Action of Vitamin B_6 HCl on Isolated Smooth Musele Organs. A. The peristaltic movements of a strip of a rabbit's small intestines immersed in Tyrode's solution at 38°C. At arrow, 0.5 ce of 2.5% solution of B_6 HCl was applied (making the concentration 1:8000). There was a brief inhibition followed

by prompt recovery.

B. The myogram of a horn of a virgin guinea pig's uterus immersed in Tyrode's solution at 38°C. At arrow, the same amount of B₆ HCl as above was added. It

resulted in a contraction.

In general, the length of the skeleton, including that of the calvarium, is somewhat less in the experimental animals than in the controls.² This is especially true of the animals treated for 3 or more months.

Along with the rather general sclerotic changes and inhibition of growth, there is evidence of resorption in the pubic bones. This commences in the body of these bones and advances along the superior and inferior ramus. These transformations may be observed after 4 or more weekly injections of 500 rat units of estrogen. If such injections are continued for 8 weeks, the superior ramus of the pubic bone becomes very short, thin and dense. In many cases in which the administration of estrogen is continued for 12 or more weeks, the entire pubic bone undergoes resorption.

Histologic examination of the skeletal tissues of representative animals in each group reveals that the epiphysial plates—especially of the bones of the knee joint—are quickly affected. The columns of hypertrophic cells are diminished in height and their matrices show premature calcification. The activity of the resting and proliferating cells is likewise inhibited, in that their rate of division is retarded. The number of cartilage cells in the epiphysial plate is diminished to a slight degree. A striking finding is the absence of the columnar arrangement of newly formed trabeculae in the zone of provisional calcification. Instead of this arrangement, one finds a disorderly agglomeration of blood vessels which have erupted into the growth plate and which may have reached the level of the proliferating cells. Around the numerous blood vessels, new bone has formed by means of osteoblastic activity in the connective tissue in the region of the zone of provisional calcification.

The new trabeculae of bone contain very small amounts of cartilaginous matrix. Instead of growing in a normal strictly vertical plane, the sheets of new bone develop in a disorderly fashion; a good many are in a horizontal plane. Most of the trabeculae fuse to form an almost solid block of bone.

The new bone rapidly fills up the medullary cavity. It advances into the diaphysial marrow cavity by spreading along the endosteal surfaces of the cortices.

It is interesting that in a group of female mice (Series 3) new bone formation was associated with resorptive phenomena. Specifically, the vessel canals were found enlarged in the cortices of the long bones and in the tables of the calvarium. Furthermore, in many places, the linings of these canals showed occasional osteoclasts.

² Zondek, B., Fol'a Clin. Orient. 1937, 1, 1,

TABLE I.

			mmatur e iee		Weekly dose	
Series	Strain	Exp.	Control	Sex	of estrogen R.U.	No. of weeks treated
1	R. Farms	6	G	Female	1000	2- 5
30	** **	G	6	Male	500	5-10
21	C_3H	7	7	Female	500	6-12
16	R. Farms	5	.5	23	500	8-20
3	",	3	3	,,	300	4-14
5	" "	ß	6	"	150	8-22

follows upon subcutaneous administrations of estradiol benzoate in sesame oil. When 1000 rat units of estrogen have been given for 3 or 4 successive weeks, an area of increased density is already observed in the sub-epiphysial plate regions of the boncs contributing to the knee joint. When the estradiol is given over the longer periods of time and in the larger doses, the dense shadow advances into, and may obliterate the medullary cavity. After administering 500 rat units of estradiol benzoate each week for 2 or 3 months, one notes an osteosclerosis of the distal third of the femur as well as of the proximal third of the tibia. This change occurs in both the male and female mice, but is somewhat less marked in the former.

The administration of 500 rat units of the estrogen per week for 4 or 5 months provokes a more generalized osteosclerosis. It may obliterate a considerable part of the medullary cavity and also cause a pronounced thickening of the cortices of all the long bones. The epiphyses, especially of the femur and tibia in the knee joint, are similarly transformed. The vertebral column also undergoes such changes. In the vertebrae, the sclerosis makes its first appearance in the zone of provisional calcification and then advances from the caudal and cephalad surfaces toward the middle of the vertebral body.

It is interesting that at no time were isolated foci of osteosclerosis observed. Furthermore, where cortical thickening is present, it seems to be the result of partial obliteration of the metaphysial or diaphysial medullary cavity. No periostitis is evident. The calvarium in general tends to become sclerotic, and usually does so after 3 or more months of administration of the estrogen in doses of 500 rat units per week. The density of the incisor teeth is somewhat increased. From some of the roentgenographs one gains the impression that the pulp spaces of the incisors and especially of the lower ones, are diminished in size.

Injections of 150 rat units of estradiol benzoate per week for 2 or 3 months, likewise causes a productive osteosclerosis, but to a lesser degree.

11386

Reversibility of Digitalis Action.*

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In Cushny's monograph on digitalis one reads the following regarding drugs of the digitalis group, "The action of these drugs is therefore reversible only in the earlier stages, and that slowly and with difficulty; later the process becomes irreversible, and the poison can be displaced neither during life nor by the use of chemical solvents after death." Again, Straub, speaking of the excised adult heart treated with digitalis, states "the final effect, after it has once set in, cannot be removed by any amount of washing. The reaction of the digitalis glucoside with the heart muscle is not reversible, and the heart is killed." This opinion is widespread.

In contrast to the idea of irreversibility of digitalis action, Kingisepp³ demonstrated the ability of electrically driven isolated frog ventricles to respond to digitalis wash-out. Arrested ventricles were made to return to their original amplitude of response as measured "by the isochoric response." In the present work further evidence as obtained from the study of the embryonic heart is given. After complete stoppage it has been possible to revive the hearts and return them to entire normality. Before presenting our data we wish to express a warm sense of appreciation for Doctor Torald Sollmann's kindly aid.

Method. Three drugs were used: ouabain in 1:100,000 dilution; tincture of digitalis in 1:100 dilution made from Reference Digitalis Powder according to U.S.P. XI; and digitoxin, as much as would dissolve in Tyrode solution in an hour's time. In each case the diluent was glucose-free Tyrode solution at pH 7.4.

The ventricle and conus of chick embryos (hereafter called ventricle), incubated for 48 hours, were excised and placed in a drop of Tyrode solution in a well slide. A coverslip was set over the well

^{*} This research was made possible by a grant from the Council on Pharmacy and Chemistry of the American Medical Association and a grant from the John and Mary R. Markle Foundation.

¹ Cushny, A. R., The Action and Uses in Medicine of Digitalis and Its Allies, p. 80, Longmans, Green and Co., New York, 1925.

² Straub, W., Stanford University Publications, Medical Sciences, 1929, 3, 45.

³ Kingiscpp, G., J. Pharm. and Exp. Therap., 1935, 55, 377.

The region of the zone of provisional calcification of the bones comprising the knee joint presented a very loose vascular osteogenic tissue in which areas of new bone were forming. The connective tissue spread for a considerable distance into the diaphysial portion of the marrow cavity. In the calvarium, the newly formed intramedullary connective tissue appeared to arise from the stroma of suture lines.

In the other series of animals, the bone-forming elements were predominant. In fact, the new bone may fill a large portion of the medullary cavity and replace a considerable amount of the lymphoid marrow. Although the most obvious effects occur in the metaphysial region of the bones of the knee joint, the upper end of the femur and the humerus, and the vertebral bodies become similarly involved, though to a lesser degree. Replacement of the lymphoid marrow by new bone occurs in the epiphyses also, and especially in those of the bones comprising the knee joint region. In these epiphyses, the subchondral areas become sclerotic.

In the articular cartilages, as in the growth plates, the cells are retarded in their activity. The cartilage cell outlines in the columnar regions are much more distinct than they would normally be. In the zone of provisional calcification, the blood vessels may advance into the calcified cartilage beyond the normal level. Interestingly enough, however, the matrix of the articular cartilage above the zone of provisional calcification does not undergo premature fibrillization or degeneration.

In general, the extent and severity of the changes in the bone and cartilage vary in accordance with the duration of the experiment and the dosage of estrogen. In the course of 4 to 5 months, the femur, tibia, and other long bones and the vertebral column, may come to show extensive replacement of the lymphoid elements in the metaphysial and diaphysial portions of the medullary cavity by newly formed bone. There is no increase in periosteal activity.

Conclusion. Injections of estradiol benzoate into immature female and male mice in the dosages listed in the table, stimulate the proliferation of new bone in the medullary cavities of certain bones and especially of the lower end of the femur and upper end of the tibia. Other bones, including the calvarium, are also affected, but to a lesser degree. The proliferation begins around the zone of provisional calcification and advances into the diaphysial portion of the bone. The changes in question can easily be recognized on roentgenographic examination. When the pubic bones are involved, they undergo in addition, more or less resorption, the extent of which depends upon the dosage of estrogen and the duration of the experiment.

desirable to remove all the Tyrode solution for fear that the ventricle would be damaged. Even small amounts can produce sufficient variation in concentration to alter appreciably time of appearance of stoppage. The ventricles were left in the ouabain 2.1 min (mean) after complete stoppage. The time when first beats occurred after washing was started averaged 7 min. These first beats were not always regular in rate. In the table the time after washing when the beats did become regular and deep is indicated under the heading "Recovery". The rate of 6 and 7 after recovery are qualified by "(irregular)". Both of these showed periods of slight acceleration followed by periods of slower rate of beat. They are not significant since controls sometimes show the same thing.

The next group of experiments involved the use of tincture of digitalis in 1:100 dilution. Tincture was made from Reference Digitalis Powder (1 cc contained 1 U.S.P. XI unit). In Table II results are summarized. The average time of ventricular stoppage was 3.4 min. After stop the preparation was left in the diluted tincture 2.7 min on the average. The first beats after wash was started appeared in 15 min (average). A difference between tincture and ouabain was noticed here however. The first ouabain beats were usually of a nature involving the entire ventricle. the tincture the first beats were usually slight "jiggles" near the end of the ventricle from which the atrium had been cut. gradually increased in depth and involved more and more of the ventricle until the entire preparation was beating. It became obvious that the tincture was more difficult to remove than ouabain since irregularities usually persisted so long as to make a second wash advisable. The mean recovery time was 46 min and the average rate of beat was 134 per min. Ventricles 4 and 5 showed

TABLE II. Ventricles in 1:100 Tincture Digitalis (U.S.P. XI).

	T) - /	3.50	772 . 7	Reco	overv
No.	Rate per min	Min in drog	First beat after wash	Time	Rate/min
1	123	2 + 3	22 min	36 min	164
2	114	2 + 3	16	55	158
3	89	$4 \div 5$	26	50	132
4	118	2 + 3	12	25	130 (irregular)
5	69	2 + 3	27	55	130
6	73	4 + 1	2	70	179
7	121	5 + 2	19	22	165
8	80	3 + 2		50	122
9	129	3 + 2	4 5	50	107
10	82	7+3	16	45	56
15	700/ 7				
Mean:	$100/\min$	3.4 + 2.7 min	15 min	46 min	134/min

to prevent excessive evaporation and the preparation was placed in a warm chamber at 38°C. After the ventricle had reached incubator temperature the rate of beat was taken. Next, Tyrode solution around the ventricle was pipetted off and replaced by a drop of the desired glucoside diluted with Tyrode solution. After being completely stopped by the glucoside, the ventricle was removed with a platinum loop to a petri dish of Tyrode solution; then to a second dish; and finally removed to a well slide containing Tyrode solution, covered, and returned to the warm chamber. This constituted what we shall call a "wash". In some cases this procedure was repeated (digitalis, wash, digitalis, wash, etc.). Rates of beat, time required for stoppage in the glucoside, time of appearance of first beats after washing was started, time required for the return of normal beating, and other pertinent data were recorded.

Observations. For purposes of comparison 10 experiments with ouabain in 1:100,000 dilution in Tyrode solution were conducted. Straub⁴ demonstrated the reversibility of this glucoside in adult frog hearts. Paff and Johnson⁵ noted its reversibility in the embryonic heart. Table I summarizes the data. In the strength used the drug stops the ventricles completely in about 3.5 min (the median). The mean is really 4.6 min for the 10 experiments but discrepancies in preparations 8 and 10 raise the average stop time considerably. Here, as in all later experiments, this is partly due to the fact that the first rate of beat was taken with the ventricle in Tyrode solution and this was replaced by the glucoside. It was not

TABLE I. Ventricles in 1:100,000 Ounbain.

				Reco	overy
No.	Rate per min	Min in drug	First beat after wash	Time	Rate/min
1	103	2 + 3*	9 min	12 min	138
<u> </u>	141	2 + 3	1	53	155
3	111	$4 \dotplus 1$	5	17	157
4	160	1 + 4	13	19	149
5	133	$\bar{3} + \bar{2}$	10	18	137
6	61	$4 \dotplus 1$	8	12	88 (irregular)
7	72	$\bar{4} \dotplus \bar{1}$	7	15	119
8	126	$\bar{9} + \bar{3}$	4	14	152
9	116	$\ddot{3} + \ddot{2}$	Ġ	8	145
10	92	$1\overset{\circ}{4}\overset{-}{+}\overset{\circ}{1}$	7	33	92
10					
Mean:	112/min	4.6 + 2.1 min	7 min	20 min	133/min

^{*}First number is time of complete stop. Second number is time left in drug after stop.

⁴ Straub, W., Biochem. Z., 1910, 28, 392.

⁵ Paff, G. H., and Johnson, J. R., Am. J. Physiol., 1938, 122, 753.

failed to elicit a response. More conclusive evidence is the fact that controls stopped by digitoxin, for example, remained absolutely quiescent to single make and break induction shocks. Furthermore the ventricles, when removed from the glucosides, continued to be affected as evidenced by the fact that they passed into extreme systole. This is especially true with tincture and digitoxin. The picture at this stage was that of a dead ventricle. Accompanying this reaction it was noted that the mesothelium at the surface of the heart seemed to become swollen beyond the amount explainable by the systole. The cells appeared rounded. We believe it also significant that the ventricles became sticky, so much so that they often adhered strongly to the glass or to the platinum loop used in transfer. When this last occurred, small portions of the heart were sometimes torn away, suggesting that the process seriously involved the muscle as well as the mesothelium.

These stages suggest marked degenerative changes. However, despite this suggestion, they are only apparent. As proof of this, ventricles stopped in tincture and in digitoxin were placed in chicken blood plasma in Carrell flasks along with controls. The results were conclusively in favor of removal. Not only did all the ventricles, both controls and glucoside-treated hearts (after recovery), beat for an observation period of 3 days but the beats, both as to rate and depth, were normal. Unless one knew which ventricles had been treated with the glucosides, it would be impossible to distinguish drugged hearts from controls. This is equally true of both physiological activity and structural integrity.

In the embryonic heart additional evidence is necessary before a reasonable hypothesis regarding the probable nature of digitalis action can be stated. The production of stickiness and rounded appearance of cells suggests an exchange of material between cell and environment accompanied by marked surface phenomena. It can be postulated, however, that no firm chemical union occurs between the digitalis and the embryonic heart muscle since the time necessary for recovery is so short.

Summary and Conclusions. 1. In embryonic ventricles stopped by ouabain, tincture of digitalis, and digitoxin the effects of the drugs can be removed by washing with Tyrode solution. 2. Of the 3 glucosides, the action of digitoxin is the most difficult of removal. Tincture, in turn, is more difficult to remove than ouabain. 3. With tincture, the process of stop, wash-out, stop, wash-out, etc., can be repeated at least 6 times in the same ventricle. 4. Digitoxin-stopped ventricles recover in blood plasma and are indistinguishable as to function and structure from controls in the same medium.

irregularities, but here again they were of a nature comparable to those seen in the controls.

The results with a single stoppage by tineture encouraged the attempt at repeated stoppage and washing with the same ventricles. The mere mechanical manipulation alone involved in this process offers a real hazard to continued activity of the embryonic ventricle. Proof that it can be successfully carried out is seen, however, in the following experiment:

Ventricle, rate, 90/min. Stopped in tineture in 2 min 40 sec. Washed 17 min. Beat again 94/min (irregular). Stop in tincture again in 45 sec. After 48 min wash the rate was 110/min (irregular). Stopped the third time in tincture in 1 min 15 sec. Washed 20 min, and beat became regular at 160/min. Stopped a fourth time in tincture in 1 min 3 sec. After 50 min of washing beat returned and was irregular. No rate was taken at this time. Stopped a fifth time in tincture in 55 sec. Wash 25 min and irregular beat returned. Again no rate taken. A sixth stop occurred in 47 min, and after recovery the rate was irregular. Mean rate was 22/min. Four other ventricles gave comparable results.

In the third group of experiments digitoxin was used. The same teehnie applied successfully with tineture and ouabain failed in 4 out of 5 attempts with digitoxin even 150 min after the single wash had begun. It was necessary to change the technic. This consisted in repeated washes at intervals of 15 min until it was apparent that complete recovery would occur. Results are summarized in Table III.

Discussion and Further Observations. The tabulated results indicate that both digitoxin and tincture as well as ouabain can be washed out of the embryonic ventricles. Of the 3, digitoxin is the most difficult to remove. Tincture is next and, as was expected, ouabain was removed with ease. The short time in which the ventricles remained in the glueosides raises the question whether or not the drug had sufficient time to act. As proof that the time is ample it was noted that mechanical manipulation of the ventricles invariably

TABLE III. Ventricles in Digitoxin.

====			No. of washe	5	Re	covery
No.	Rate per min	Min in drug	(15-min intervals)	First beat after wash	Time	Rate/min
1 2 3 4 Mean:	104 99 90 116	$ \begin{array}{r} 5 + 2 \\ 9 + 2 \\ 13 + 2 \\ 2 + 3 \\ \hline 7.2 + 2.2 \\ \end{array} $	8 6 7 6 ————————————————————————————————	64 min 80 82 56 	142 min 85 92 119 —	111 133 115 90 — 112/min

All 3 types of Shigella mentioned above grew very well on the egg membrane. From one to 10 microörganisms were found to be enough to obtain growth on the membrane. If the growth was only scarce, no gross changes were seen on the membrane. If infection was intense, infiltrates varying from small greyish spots to purulent exudates, sometimes also small hemorrhages, were seen. The degree of this reaction was roughly proportional to the amount of bacteria implanted.

Generalized infection could be produced with each strain. The amounts necessary for this purpose varied from 50 to 50,000 microorganisms. They were fairly constant for each strain. Where generalized infection took place, the microörganisms could be recovered from the heart blood and the organs of the embryo, and the embryos died in 24 to 72 hours, exceptionally also on the fourth day after infection.

Smooth and partially rough cultures were infective to the same degree. However, perfectly rough variants were found to be devoid of invasive power, and thus did not kill the embryos. With our Flexner strains, there were several observations of the reversion from partially rough to smooth after egg passage. No such reversion was observed with Sonne strains.

In many cases quick disintegration of the contents of the eggs followed death. This was especially marked in cases infected with Sonne strains. Where the dead embryos were well preserved, no characteristic findings were noted upon inspection.

We are planning a histological study of both membranes and embryos in order to obtain information on the host's reaction and on possible special localizations of the infective agent.

It was found also possible to cause a lethal infection by injection of dysentery bacilli into the yolk sac of the embryo. In this case, generalized infection takes place within 6 hours.

11387

Infection of the Developing Chick Embryo with Dysentery Bacilli.

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The experimental approach to the problems connected with microorganisms of the Shigella group is greatly hampered by the lack of a convenient laboratory animal sensitive to this type of infection. The purpose of this paper is to report on our experiences with the infection of chick embryos with dysentery bacilli, which we hope may contribute toward the alleviation of this difficulty.

Goodpasture and his coworkers have studied bacterial infection in the chick embryo and have investigated the reactions of this new host.^{1, 2, 3} There are also some reports from other laboratories on

applications of this method to problems of a similar scope. 4-7

For our study we have used 2 strains of Shigella dysenteriae ("Shiga bacillus"), 7 of Shigella paradysenteriae ("Flexner bacillus"), and 7 of Shigella sonnei. All were carefully controlled for their microscopic, cultural and serological properties.

We found fertile eggs incubated 9 to 10 days most favorable for our purposes. We followed, in the main, Goodpasture's procedure.^{8,9} If the usual precautions of bacteriological work are applied, the danger of contamination of the eggs is certainly not greater than that of agar plates.

The number of viable microörganisms introduced was checked by plate count of 10⁻⁷ dilutions of the broth culture. On the average the broth cultures contained 500,000,000 microörganisms per ml. The eggs were infected by dropping 0.1 ml amounts of broth diluted serially 10-fold with saline solution upon the chorio-allantoic membrane. The infected eggs were incubated at 37.5°C.

¹ Goodpasture, E. W., and Anderson, K., Am. J. Path., 1937, 13, 149.

² Goodpasture, E. W., Am. J. Hyg., 1938, 28, 111.

³ Buddingh, G. J., and Polk, A. D., J. Exp. Med., 1939, 70, 485, 489, 511.

⁴ Morrow, G., and Berry, G. P., J. Bact., 1938, 38, 38.

⁵ Morrow, G., Syverton, J. T., Stiles, W. W., and Berry, G. P., Science, 1938, 88, 385.

⁶ Moore, M., Science, 1939, 89, 1939.

⁷ Sterzi, G., and Staudacher, V., Giorn. ital. di dermat. e sif., 1939, 17, 4.

⁸ Goodpasture, E. W., and Buddingh, G. J., Am. J. Hyg., 1935, 21, 319.

⁹ Burnet, F. M., Med. Res. Council, Spec. Rep. Ser. 220, London, 1936.

All 3 types of Shigella mentioned above grew very well on the egg membrane. From one to 10 microörganisms were found to be enough to obtain growth on the membrane. If the growth was only scarce, no gross changes were seen on the membrane. If infection was intense, infiltrates varying from small greyish spots to purulent exudates, sometimes also small hemorrhages, were seen. The degree of this reaction was roughly proportional to the amount of bacteria implanted.

Generalized infection could be produced with each strain. The amounts necessary for this purpose varied from 50 to 50,000 microorganisms. They were fairly constant for each strain. Where generalized infection took place, the microörganisms could be recovered from the heart blood and the organs of the embryo, and the embryos died in 24 to 72 hours, exceptionally also on the fourth day after infection.

Smooth and partially rough cultures were infective to the same degree. However, perfectly rough variants were found to be devoid of invasive power, and thus did not kill the embryos. With our Flexner strains, there were several observations of the reversion from partially rough to smooth after egg passage. No such reversion was observed with Sonne strains.

In many cases quick disintegration of the contents of the eggs followed death. This was especially marked in cases infected with Sonne strains. Where the dead embryos were well preserved, no characteristic findings were noted upon inspection.

We are planning a histological study of both membranes and embryos in order to obtain information on the host's reaction and on possible special localizations of the infective agent.

It was found also possible to cause a lethal infection by injection of dysentery bacilli into the yolk sac of the embryo. In this case, generalized infection takes place within 6 hours.

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Infection of the Developing Chiek Embryo with Dysentery Bacilli.

A. J. WEIL AND J. A. VOLENTINE.

From the Department of Biological Research, Lederle Laboratories, Inc., Pearl River, N. Y.

The experimental approach to the problems connected with microorganisms of the Shigella group is greatly hampered by the lack of a convenient laboratory animal sensitive to this type of infection. The purpose of this paper is to report on our experiences with the infection of chick embryos with dysentery bacilli, which we hope may contribute toward the alleviation of this difficulty.

Goodpasture and his coworkers have studied bacterial infection in the chick embryo and have investigated the reactions of this new host.^{1, 2, 3} There are also some reports from other laboratories on applications of this method to problems of a similar scope.⁴⁻⁷

For our study we have used 2 strains of Shigella dysenteriae ("Shiga bacillus"), 7 of Shigella paradysenteriae ("Flexner bacillus"), and 7 of Shigella sonnei. All were carefully controlled for their microscopic, cultural and serological properties.

We found fertile eggs incubated 9 to 10 days most favorable for our purposes. We followed, in the main, Goodpasture's procedure.^{8, 9} If the usual precautions of bacteriological work are applied, the danger of contamination of the eggs is certainly not greater than that of agar plates.

The number of viable microörganisms introduced was checked by plate count of 10⁻⁷ dilutions of the broth culture. On the average the broth cultures contained 500,000,000 microörganisms per ml. The eggs were infected by dropping 0.1 ml amounts of broth diluted serially 10-fold with saline solution upon the chorio-allantoic membrane. The infected eggs were incubated at 37.5°C.

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⁸ Goodpasture, E. W., and Buddingh, G. J., Am. J. Hyg., 1935, 24, 319.

⁹ Burnet, F. M., Med. Res. Council, Spec. Rep. Ser. 220, London, 1936.

studies indicated that the particular strain of dysentery organisms used in this investigation grew more luxuriantly in a dextrose-free yeal medium, the carbohydrate was omitted from the broth in which this organism was studied. The medicated culture media were autoclaved at 10 lb for 10 minutes and upon cooling 1 cc of an 18-hour broth diluted culture of one of the several organisms was added to each drug-broth and drug-free control broth medium.

The inoculated solutions were incubated at 37°C for 23-25 hours. In order to estimate the degree of bacteriostasis at this time, the following procedures were carried out. One-tenth and 1.0 cc of appropriate broth dilutions were placed in each of 2 petri dishes and melted and cooled (45°C) veal dextrose agar added. contents were mixed thoroughly by swirling and the agar allowed to solidify. The plates were incubated at 37°C for 72 hours at which time the growing colonies were counted.

Results. In Table I are presented the results of the in vitro effects

TABLE I. Comparison of In Vitro Effects of the Several Compounds upon E. typhosa, E. coli, and S. dysenteriæ.

		10 mg%	concentra	ations. 23	25 hrs. 37	°C. Orga	nisms/cc.
	noculum acteria/cc	Sulfanil- amide	Sulfa- pyridine	Sulfa- thiazol	Sulfa- methyl- thiazol	Sulfa- phenyl- thiazol	Control
E. typhosa "Rawling", "B"	45 75	420 M 330 M	125,000 450	35,000 0	20,000 250	320,000	570 M 670 M
E. typhosa "Rawling" "M	, 40	500 M	780 M	110 M	100 M	610 M	660 M
	80	725 M	490 M	120 M	40 M	410 M	590 M
E. typhosa "Hopkins"	40	520 M	670 M	340 M	350 M	530 M	600 M
	60	450 M	660 M	40 M	135 M	370 M	490 M
E. typhosa	70	620 M	460 M	370 M	410 M	640 M	930 M
No. 305	60	630 M	375 M	215 M	170 M	380 M	650 M
E. typhosa	70	620 M	750 M	370 M	440 M	580 M	830 M
No. 1006	60	750 M	620 M	230 M	290 M	620 M	890 M
E. coli	17	368 M	28 M	3 M	6 M	46 M	754 M
	90	740 M	1 M	490,000	720,000	250,000	1.1 B
E. coli	23	1.1 B	112 M	3.3 M	4.7 M	32 M	1.2 B
	75	1 B	40 M	4 M	17 M	86 M	1.2 B
S. dysenteriæ	4	484 M	308 M	130 M	122 M	145 M	425 M
	14	595 M	342 M	112 M	144 M	122 M	518 M

 ⁼ Compound not tested.
 0 = No growth in plate inoculated with 0.1 ec. of the undilnted drug broth solution or dilutions thereof.

M = Million.

B = Billion.

11388

Bacteriostatic Effects of Sulfanilamide, Pyridine and Thiazol Derivatives upon Colon-Typhoid-Dysentery Group.*

C. A. LAWRENCE. (Introduced by O. W. Barlow.)

From the Research Laboratories of the Winthrop Chemical Company, Inc., Rensselaer, New York.

Chemotherapeutic studies of a series of thiazol derivatives of sulfanilamide under conditions of experimental infections in animals with beta hemolytic streptococci (strain C203), Staphylococcus aureus, and pneumococci Types I, II and III as reported by Fosbinder and Walter, McKee, et al., and Barlow and Homburger, suggested that at least 2 of these compounds merited careful clinical examination. A comparison of the in vitro effects of these compounds on the above organisms indicated that they were superior to either sulfanilamide or sulfapyridine (Lawrence).

The present investigation was undertaken to determine the comparative in vitro effects of the thiazol compounds (sulfathiazol, sulfamethylthiazol and sulfaphenylthiazol) with those of sulfanilamide and sulfapyridine upon additional groups of organisms, namely, the gram negative bacilli representative of the colon-typhoid-dysentery group. These included 5 strains of Eberthella typhosa, one culture of which had recently been isolated from a typhoid patient (No. 1006), 2 cultures each of Escherichia coli and Aerobacter aerogenes, and one strain each of Salmonella paratyphi, S. schottmuelleri, S. snipestifer, S. psittacosis, S. enteritidis, Shigella dysenteriae and Proteus vulgaris.

Method. Ten mg % drug-broth solutions were prepared by adding the dry powders to 100 cc quantities of veal dextrose broth of pH 7.4 and containing bacto peptone.† Since preliminary cultural

^{*} The nuthor wishes to express his appreciation to Dr. J. J. Clemmer for many of the cultures used in this study.

¹ Fosbinder, R. F., and Walter, L. A., J. Am. Chem. Soc., 1939, 61, 2033.

² McKec, G. M., Rake, G., Greep, R. O., and Van Dyke, H. B., Proc. Soc. Exp. Biol. And Med., 1939, 42, 417.

³ Barlow, O. W., and Homburger, E., Proc. Soc. Exp. Biol. and Med., 1939, 42, 792.

⁴ Barlow, O. W., and Homburger, E., Proc. Soc. Exp. Biol. and Med., 1940, 13 317

⁵ Lawrence, C. A., PROC. Soc. Exp. BIOL. AND MED., 1940, 43, 92.

[†] A few crystals remaining at the bottom of the flask containing the sulfaphenylbroth solution, after autoclaving and cooling to 37°C, indicated this solution to be slightly supersaturated.

In a more recent publication Long and Bliss⁷ also found that the unsubstituted thiazol derivative was equal to, or slightly superior to, sulfanilamide and to sulfapyridine in its bacteriostatic action upon cultures of *E. coli* and *B. proteus* in broth.

Conclusions. On the basis of in vitro studies sulfathiazol and sulfamethylthiazol have been found to be somewhat more effective than sulfapyridine, sulfaphenylthiazol and sulfanilamide in their in vitro effects upon bacteria representative of the colon-typhoid-dysentery group. In general, the unsubstituted thiazol derivative appears to be the most active compound, being followed in decreasing order of effectiveness by sulfamethylthiazol, sulfapyridine, sulfaphenyl thiazol and sulfanilamide.

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Changes Produced by Desoxycorticosterone Overdosage in the Rat.*

HANS SELYE AND CHRISTIANE DOSNE.

From the Department of Anatomy, McGill University, Montreal, Canada.

Kuhlmann, et al., found recently that dogs chronically treated with very high doses of desoxycorticosterone acetate show definite signs of damage and reveal blood chemical changes which appear to be the opposite of what is seen in adrenal insufficiency. Thus they noted "... a striking decrease in serum potassium, a slight increase in serum sodium, a slight decrease in serum protein and non-protein nitrogen...". In this connection it appears of interest to mention our experiments in the rat which indicate that treatment with as high a dose as 10 mg of desoxycorticosterone acetate daily given for 20 days fails to produce any externally visible signs of damage and that, contrary to expectations, the blood chlorides prove to be consistently low. Meanwhile we have no explanation for the fact that although desoxycorticosterone prevents hypochloremia in the adrenalectomized rat, chronic overdosage with this

⁷ Long, P. H., and Bliss, E. A., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 324.

^{*} The expenses of this investigation have been defrayed in part from a grant in aid received from the Schering Corporation of Bloomfield, N. J. The desoxy-corticosterone acetate used in our experiments has been kindly supplied by Drs. G. Stragnell and E. Schwenk of the Schering Corporation.

¹ Kuhlmann, D., Ragan, C., Ferrebee, J. W., Atchley, D. W., and Loeb, R. F., Science, 1939, 90, 496.

of the several compounds upon E. typhosa, E. coli and S. dysenteriae. While the differences in the degree of bacteriostasis produced by most of these compounds under these experimental conditions are not striking, there is a definite trend which indicates a greater inhibitory effect on the part of sulfathiazol and sulfamethylthiazol than that of the other compounds.

A comparison of the effects of the compounds upon the Salmonella, Acrobacter and Proteus vulgaris organisms is given in Table II. In general sulfathiazol and sulfamethylthiazol were again found to be somewhat more effective than sulfapyridine, and distinctly more active than sulfaphenylthiazol and sulfanilamide in their bacteriostatic actions upon the organisms studied.

These findings, in part, confirm the results obtained by Helmholz⁶ who, under different experimental conditions, was able to show that sulfathiazol and sulfamethylthiazol were more effective than sulfamilamide and sulfapyridine in their in vitro actions upon many of the organisms associated with urinary infections.

Comparison of the In Vitro Effects of the Several Compounds upon Salmonella,

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	10 mg% concentrations. 23-25 hrs. 37°C.						
Organism	Inoculum Bacteria/ce	Sulfanil- amide	Sulfa- pyridine	Sulfa- thiazol	Sulfa- methyl- thiazol	Sulfa- phenyl- thiazol	Control
S. paratyphi	110	1.3 B*	600 M	770,000	7 M	810 M	1.1 B
	155	1 B	520 M	260 M	225 M	520 M	1.1 B
S. schottmuell	cri 160	1.6 B	650 7t	8 M	115 M	860 M	1.6 B
	185	1.5 B	730 7t	460 M	690 M	1.2 B	2 B
S. suipestifer	95	180 M	1,200	150	400	1,150	890 M
	100	340 M	80	40	270	10	740 M
S. psittacosis	70	380 M	350 M	15 M	60 M	700 M	580 M
	75	610 M	278 M	67 M	195 M	500 M	725 M
S. enteritidis	120	1 B	37 M	270,000	600,000	680 M	1.2 B
	47	690 M	630 M	50 M	105 M	400 M	720 M
A. acrogenes	17	446 M	286 M	120 M	132 M	355 M	512 M
	26	425 M	224 M	94 M	160 M	376 M	489 M
A. acrogenes	90	160 M	74 M	35 M	78 M	83 M	940 M
	150	1.4 B	430 M	60 M	90 M	900 M	1.2 B
Proteus vulgari	s 37	460 M	13 M	1.5 M	1.5 M	15 M	290 M
	5	472 M	220 M	151 M	135 M	22 M	460 M

^{*}See legend under Table I.

⁶ Helmholz, H. F., Proc. Staff Meetings, Mayo Clinic, 1940, 15, 65.

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TABLE II.

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Acrobacter and Proteus vulgaris Organisms.

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TABLE I. Changes in Blood Chemistry and Adrenal Weight Induced by Desoxycorticosterone Overdosage.

Treatment	No. and sex of animals	ex 13 Body wt	Blood chlorido	Blood chlorido Blood glucoso	Adrenal wt
Desoxycorticosterono acotato 2 mg in 0.1 ce oil/day for 20 days Oil contects	99	157 (124-185) 2 100 (74-135)	335 (326-349)	90 (92-107) 86 (71-103)	40 (26-58)
0.1 cc/day for 20 days	99	\$ 159 (142-172) \$ 108 (73-145)	429 (425-431) 430 (420-443)	95 (83-103) 95 (88-107)	35 (29-44) 42 (38-53)
Desoxycorticosterono acetato 10 mg in 0.4 ee oil/day for 20 days Oil one can	99	\$ 159 (147-174) \$ 152 (103-131)	380 (369-404) 381 (374-410)	70 (67-79) 86 (75:96)	19 (15-21) 22 (10-28)
0.4 ce oil/day for 20 days	99	\$ 167 (147-178) \$ 116 (106-131)	430 (421-456)	82 (75-92)	38 (33-43)
Desoxycorticosterono acetato 10 mg in 0.4 cc oil/day for 20 days Cholosterol controls	ဗ	3 150 (140-159)	334 (322-351)	85 (71-96)	
10 mg in 0.4 ec oil/day for 20 days	9	150 (140-160)	400 (380-416)	86 (71-96)	40 (35-43)

compound decreases the blood chloride concentration in normal animals. Our experiments clearly indicate, however, that this is the case. The blood chlorides were determined by the Van Slyke method and are expressed in mg of NaCl/100 cc of blood. Blood sugars (Hartmann-Shafer-Somogyi's method) are expressed in mg of glucose/100 cc and the adrenal weight in mg. White Wistar rats were used for all experiments. Desoxycorticosterone was administered once daily by subcutaneous injections in peanut oil, the last injection being given 24 hours before autopsy. All animals were fasted for 24 hours before the determinations. In Table I, which summarizes our findings, the average values are given with the range of variations in brackets.

As the table indicates, the blood chlorides are significantly decreased in each case while the blood sugar does not appear to be affected. The absence of hypoglycemia indicates that overdosage with desoxycorticosterone acetate does not elicit all the symptoms of adrenal insufficiency. Gross estimations of the blood volume of our animals show that a decrease in the amount of the circulating blood-also characteristic of adrenal deprivation-likewise fails to occur in case of desoxycorticosterone overdosage. We mention this particularly because, contrary to the statement of Kendall,2 who claimed that desoxycorticosterone and its acetate cause no significant adrenal atrophy-and thereby differ from corticosterone and compound E-we noted in agreement with our previous findings3 that the adrenals became very atrophic. It should be stated, however, that Kendall used relatively small doses of desoxycorticosterone and that from his findings, it appears that corticosterone and his compound E are even more active in causing adrenal involu-

Summary. Experiments on the rat indicate that chronic treatment with desoxycorticosterone acetate in daily doses of up to 10 mg does not lead to any significant external signs of damage but causes marked hypochloremia and adrenal atrophy. Since the blood sugar and blood volume is not significantly influenced by this treatment, the hypochloremia cannot merely be regarded as a sign of general adrenal insufficiency resulting from the atrophy of the adrenal cortex unless one assumes that the compound interferes specifically with the chloride regulating function of these glands. It should be emphasized, however, that even such doses of desoxycorticosterone acetate which do not suffice to cause significant adrenal atrophy produce definite hypochloremia.

² Kendall, Edward C., Proc. Am. Soc. Biol. Chem., New Orleans, 1940.

³ Selye, Hans, Canad. Mcd. Assn. J., 1940, 42, 113.

TABLE I. Changes in Blood Chemistry and Adrenal Weight Induced by Desoxycorticosterone Overdosage.

	(106-131)	(1.17-178)	(103-131) 381 ((147-174) 380 (73-145) 430	142-172) 429 (74-135) 335 (124-185) 380 (No. and sex of animals Body wt Blood chloride
8 6 & 150 (140-159) 334 (322-351)	6 3 150 (140-159)	6 & 150 (140-159)	6 \$ 167 (147-178) 6 \$ 116 (106-131) 6 \$ 150 (140-159)	(103-131) (147-178) (106-131) (140-159)	6 \$ 159 (147-174) 6 \$ 122 (103-131) 6 \$ 167 (147-178) 6 \$ 116 (106-131) 6 \$ 150 (140-159)	6 \$ 108 (73.145) 6 \$ 159 (147.174) 6 \$ 122 (103.131) 6 \$ 167 (147.178) 6 \$ 167 (147.178) 6 \$ 160 (140.159)	6 \$ 159 (142-172) 6 \$ 108 (73-145) 6 \$ 159 (147-174) 6 \$ 167 (147-178) 6 \$ 116 (106-131) 6 \$ 150 (140-159)	6 \$ 100 (74-135) 6 \$ 159 (142-172) 6 \$ 108 (73-145) 6 \$ 122 (107-174) 6 \$ 167 (147-178) 6 \$ 116 (106-131) 6 \$ 150 (140-159)	6 \$ 157 (124.185) 6 \$ 100 (74.135) 6 \$ 159 (142.172) 6 \$ 108 (73.145) 6 \$ 159 (147.174) 6 \$ 167 (147.174) 6 \$ 167 (147.178) 6 \$ 6 \$ 167 (147.178) 6 \$ 6 \$ 167 (147.178) 7 \$ 169 (140.159)
		(106-131)		(105-131) 381 ((147-178) 430 ((106-131) 447 ((147-174) (103-131) (147-178) (106-131)	(73-145) 430 (147-174) 380 (103-131) 381 (147-178) 430 (106-131) 447 ((142-172) 429 (73-145) 430 (147-174) 380 (103-131) 430 (166-131) 447	(74-135) 335 ((142-172) 429 ((73-145) 430 ((147-174) 380 ((103-131) 381 ((147-178) 430 ((74-135) 335 (74-135) 335 (74-175) 429 (73-145) 430 (147-174) 380 (147-178) 430 (147-178) 447 (106-131)

may serve as an index of testicular activity. The data for each group were massed and analyzed statistically by Fisher's method. A P less than 0.05 was held as the criterion for probable significance of any difference observed between the test and control groups.

Results. Neither initial nor final body weights of the test group (32.1 g and 73.8 g respectively) showed any significant difference from the corresponding control weights (30.9 g and 70.8 g respectively).

The seminal vesicles of the treated animals averaged 33.5 mg and were significantly heavier than the corresponding mean of 22.5 mg for the controls (P was less than 0.01). The weight of the testes of the treated group averaged 546 mg as compared to a mean of 563 mg for the controls. The difference of 17 mg was only a 3% difference and was statistically insignificant (P was greater than 0.1).

Microscopically, the testes of all animals were normal. Spermatogenesis was incomplete in both treated and control groups. The epididymides of the treated group, however, contained numerous actively mitotic immature spermatocytes within their tubules. In the normals, this was only an occasional finding.

Discussion. From this study it may be seen that an androgenically potent dose of testosterone propionate as judged by its stimulating effect upon the seminal vesicles, has spared the testis the depression heretofore described. Biddolph has also observed a practically negligible depression with 2γ doses administered daily from birth to 31 days of age. From his report, however, one was at a loss to know whether his dosage was androgenically potent otherwise. The presence of immature spermatozoal forms in the tubules of the epididymis indicates that testosterone propionate stimulates proliferation of the germinal epithelium without hastening maturation. In these respects it acts like the gonadotropic hormones. It differs from the gonadotropins in that it fails to stimulate the interstitial tissue.

Conclusions. Testosterone propionate injected subcutaneously in 10 γ doses daily for 10 days to albino rats from 22 to 32 days proved to be androgenically potent causing a "probably significant" enlargement of the seminal vesicles. The testes, however, were neither depressed in weight nor were their histological pictures altered. Proliferation of the germinal epithelium was hastened.

⁶ Rubinstein, H. S., Endocrinology, 1938, 23, 171.

Fisher, R. A., Statistical Methods for Research Workers, Oliver and Boyd, London, 1936.

11390

Effect of Small Doses of Testosterone Propionate on the Testis.*

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Practically all reports concerning the effect of testosterone propionate on the testis stress the depressing effect of this hormone.^{1, 2, 3} Biddolph,⁴ using doses as small as 2 γ daily in animals treated from birth has, however, reported that the testes of his treated animals were practically unaffected. Since this author did not simultaneously publish any data concerning the accessory sex organs of his treated group it is conceivable that the dose which he used was so small that it not only failed to affect the testes but was also devoid of any androgenic effect. For this reason the following study was undertaken.

Thirty-six male albino rats (Mus norvegicus, var. albus) of Wistar Institute strain were divided into 2 groups consisting of 16 test animals and 20 littermate brother controls respectively. The test animals were given daily subcutaneous injections of 10 y of testosterone propionate in sesame oil (Perandren) for 10 days beginning on the 22nd day of life. Except for these injections all animals were similarly treated, being fed on a diet of Purina dog chow daily and green vegetables twice weekly. Water was constantly present. The controls received no injections, since the solvent used (sesame oil) has been shown to have no sex-stimulating qualities.5 The animals were weighed before the first injection and at the time of sacrifice which was carried out by carotid incision under ether anesthesia at 32 days of agc. Testes devoid of epididymides and seminal vesicles were excised, weighed, fixed in Bouin's solution and stained by haemotoxylin and eosin for microscopic study. The epididymides were excised and similarly prepared for microscopic study. This was done because it had previously been noted that the epididymis through its tubular contents

^{*} The authors appreciate the aid of the Ciba Pharmaceutical Products Company, Inc., for partially defraying the expenses of this study and for supplying the testosterone propionate (Perandren) used.

¹ Moore, C. L., and Price, D., Anat. Rec., 1938, 71, 59.

² Korenchevsky, V., and Hall, K., Brit. Mcd. J., 1939, 1, 4. ³ Korenchevsky, V., Dennison, M., and Hall, K., Biochem. J., 1937, 31, 1434.

⁴ Biddolph, C., Anat. Rec., 1939, 73, 447.

⁵ Stone, C. P., J. Comp. Psychol., 1938, 25, 445.

A definite quantitative factor was observed with respect to the number of nerve fibers exposed to the virus. In 3 cases in which only the nerve to the hamstring muscles was cut and the cut end then soaked with virus, no paralysis resulted. Inoculation of the cervical sympathetic trunk by the same method in 2 animals failed also. In one case in which the central cut end of the vagus nerve was immersed in virus for 3 minutes, paralysis did not result, whereas in another animal in which the vagus nerve was similarly soaked for 15 minutes, neck paralysis and hoarseness resulted after an incubation period of 17 days. Inoculation of the hypoglossal nerve by this method was also successful in one case, the incubation period being 4 days. Apparently then, the numbers of nerve fibers exposed, and perhaps the size of the fibers and the length of time immersed are significant quantitative factors. In any case, only a small quantity of virus suspension is necessary.

The significance and advantages of the above described method of inoculation of peripheral nerves are obvious. When sufficient numbers of axonal processes of nerve cells are exposed to small amounts of virus at a definite point in the peripheral nerve, paralysis results. Because of the definitely known location at which the virus first comes into contact with the nerve fibers, because of the assurance that all of the fibers of the nerve are placed in contact with the virus, and because in any particular nerve the numbers and sizes of fibers thus exposed can be determined, this method offers possibilities for quantitative determinations of virus potency, of speeds of transmission of viruses along nerves, and of other important but not easily obtained data.

11391 P

An Effective Method of Intraneural Inoculation of Poliomyelitis Virus.*

DAVID BODIAN AND HOWARD A. HOWE.

From the Department of Anatomy, Johns Hopkins University.

The uncertainty of results obtained by most investigators with intraneural injections of polionyelitis virus has limited the use of this method of inoculation for inducing experimental polionyelitis. Neveretheless, if the virus of polionyelitis is truly neurotropie, one would expect that intraneural inoculation would be as effective as intracerebral inoculation, and simpler, if the virus could actually be made to come into contact with numerous nerve fibers, rather than being forced along and between connective tissue sheaths within the peripheral nerve. This of course is strongly suggested by the work of Fairbrother and Hurst, who showed that trauma during intraneural injection facilitates "takes" by this method of inoculation.

Going one step farther, and with the knowledge that during the first few days after nerve section the nerve cells with axons cut are more susceptible to the virus than normal cells, it was decided to determine whether simple section of a peripheral nerve and immersion of the central stump in virus suspension for a few minutes was sufficient to produce poliomyclitis. This method, which involves no mechanical injection pressure, and which places the virus in contact with the protoplasm of every axon in the nerve, was found to be highly successful in producing poliomyclitis. When a large nerve, such as the sciatic nerve, was used, this method of inoculation was invariably successful with two strains of known potency, the MV and Wfd³ strains.

In 9 Rhesus monkeys the sciatic nerve was sectioned with sharp scissors peripheral to the sciatic notch or at the mid-thigh level, and the central cut end then soaked in as little as 0.1 cc of 20% virus suspension for several minutes. Poliomyelitis resulted after an incubation period of 4-6 days. In 5 cases the leg on the side of inoculation was completely paralyzed on the fifth day; the opposite leg was usually paralyzed completely also during the course of the same day.

^{*} This work was supported by a grant from the Commonwealth Fund.

¹ Fairbrother, R. W., and Hurst, E. W., J. Path. and Bact., 1930, 33, 17.

² Howe, H. A., and Bodian, D., PROC. Soc. EXP. BIOL. AND MED., 1939, 42, 346.

³ Trask, J. D., and Paul, J. R., J. Bact., 1936, 31, 527.

quently rise above 100 mm Hg, falling later to lower levels; in 2 others it rose for a short time above 90. Changes comparable in magnitude occurred in the systolic pressure. No effect was noticed until the injection had been given 5 to 15 minutes, when a spontaneous fall was seen. The blood pressure of five rats followed 1, 5 and 14 days remained low. An inactive preparation of the enzyme gave no effect.

Variable results were seen in *normal* rats. In 2, the diastolic pressure fell significantly. In the remainder the change was of smaller magnitude or a distinct rise was noticed (Table II).

Five animals operated upon failed to develop hypertension, and injection of the enzyme into them gave inconsistent results similar to those found in normal rats.

Injection of the enzyme appeared to have no toxic effect upon the animals.

The fact that this enzyme acts consistently as a depressor in hypertensive rats and has a variable effect in normal ones suggests that some substance common to the former is changed. It is possible, from the specificity of the enzyme for phenolic configurations, that the substance contains one or more of these chemical groups. No adequate interpretation of these results can, however, be made now,

TABLE II (Normal Animals).

Diastolic Blood Pressure mm Hg. Before and After the Injection of Tyrosinase.

Rat No.	Dose, cc	Control	15 min after injection	30 min after injection	Change a	
22	0.5	90	121	110	+20	102-45 min later
23	0.5	60	70	60	. 0	6061 ", ",
24	0.5	75	70	79	+ 4	79-64 "
26	0.8	115	95	80	35	
27	0.5	100	100	100	0	
28	0.75	70	50	52	18	5555 '' ''
29	0.7	94	70	60	34	
31	1.0	114	100	100	-14	
32	0.8	85	118	114	+29	
33	0.5	108	110	87	21	11217 days "
34	0.5	80	86	92	+12	106 9 " "
35	0.5	105	126	120	+15	110-60 min later
36	0.5	100	112	112	+12	120-45 " "
37	0.5	136	144	136	0	
40	0.5	80	65	65	15	
41	0.5	122	126	122	0	
42	0.5	102	104	98	4	9845 " "
43	0.5	102	110	114	+12	110-60 ""
45	0.5	85	86	82		
80	1.0	80	65	87	$\frac{-3}{+7}$	
		M = 95	M = 96	M = 93	$M = -\frac{5}{5}$ $\sigma = 52$	

11392 P

Effect of Tyrosinase on Blood Pressure of Hypertensive Rats.

HENRY A. SCHROEDER. (Introduced by A. E. Colm.)

From the Hospital of the Rockefeller Institute for Medical Research, New York.

Recent studies on the action of pressor substances in experimental arterial hypertension make it probable that some pressor material liberated by diseased kidneys is responsible for the elevation of blood pressure. Because this pressor substance may be a relatively simple amine, an attempt has been made to learn the action of enzymes capable of altering certain amines, upon the blood pressure of hypertensive and of normal animals. Rats were made hypertensive (1) by partial constriction of one renal artery, (2) by unilateral hydronephrosis, and (3) by unilateral renal injury, the other kidney remaining healthy. Tyrosinase, obtained from Professor J. M. Nelson of Columbia University, was injected intravenously into 37 animals. Blood pressure was measured by a Hamilton manometer, the needle of which was inserted into the femoral artery, and records made for 40 to 60 minutes thereafter. In a few instances another measurement of blood pressure was made several days later.

In every ease the diastolic pressure of the hypertensive animals fell 30 mm Hg or more (Table I), and in no case did it return to the initial level. Indeed in only one animal (Rat G 104) did it subse-

TABLE I (Abnormal Animals).

Dinstolie Blood Pressure mm Hg. Before and After the Injection of Tyrosinase.

Diastone.	2.004 2.00		,			
Rat No.	Dosc, ec	Control	15 min nfter injection	30 min after injection	Change at 30 min	Subsequent level
H 93 H 94 H 95 H 97 H 100 G 104 G 113 G 115 G 127 G 121 G 122 G 123 G 140 I 19 I 22	0.5 1.0 0.4 1.0 0.5 0.8 0.5 1.0 0.8 0.5 0.6 1.0 0.75	120 110 102 126 112 134 110 120 132 110 110 128 128 112	50 81 50 93 52 86 65 102 86 104 100 90 75 60	70 65 65 85 82 100 69 74 92 80 72 88 68 68	60 52 42	85—24 hr later 82—70 min '' 70— 2 wks '' 60— 5 days '' 92— 2 wks '' 82—98 min '' 82—75 '' '' 62—75 '' '' 100—17 days '' 85—75 min ''
		M=120	M = 80	M = 78	$ \begin{array}{l} 42 \\ \sigma = 7.8 \end{array} $	

quently rise above 100 mm Hg, falling later to lower levels; in 2 others it rose for a short time above 90. Changes comparable in magnitude occurred in the systolic pressure. No effect was noticed until the injection had been given 5 to 15 minutes, when a spontaneous fall was seen. The blood pressure of five rats followed 1, 5 and 14 days remained low. An inactive preparation of the enzyme gave no effect.

Variable results were seen in normal rats. In 2, the diastolic pressure fell significantly. In the remainder the change was of

smaller magnitude or a distinct rise was noticed (Table II).

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The fact that this enzyme acts consistently as a depressor in hypertensive rats and has a variable effect in normal ones suggests that some substance common to the former is changed. It is possible, from the specificity of the enzyme for phenolic configurations, that the substance contains one or more of these chemical groups. No adequate interpretation of these results can, however, be made now,

TABLE II (Normal Animals).

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22	0.5	90	121	110	+20	102-45	min	later
23	0.5	60	70	60	0	60 - 61	"	,,
24	0.5	75	70	79	十 4	79 - 64	"	77
26	0.8	115	95	80	-35			
27	0.5	100	100	100	0			
28	0.75	70	50	52	18	5555	23	"
29	0.7	94	70	60	34			
31	1.0	114	100	100	14			
32	0.8	85	118	114	+29			
33	0.5	108	110	87	-21	112-17	day	s ,,
34	0.5	80	86	92	+12	106 9	,,	,,
35	0.5	105	126	120	+15	11060	min	late
36	0.5	100	112	112	+12	120-15	"	,,,
37	0.5	136	144	136	0			
40	0.5	89	65	65	15			
41	0.5	122	126	122	0			
42	0.5	102	104	98	4	9845	"	y 1
43	0.5	102	110	114	+12	11060	"	3 1
45	0.5	85	86	82	3			
80	1.0	80	65	87	+ 7			
		M = 95	M = 96	M = 93	$M = -\frac{1}{\sigma}$ $\sigma = 59$			

nor can consideration of these effects be applied to any animals save rats with unilateral renal disease until further studies, now in progress, indicate that similar action upon blood pressure takes place on the use of such enzymes in other mammals.

Summary. The injection of tyrosinase into hypertensive rats consistently and markedly lowered the blood pressure, this effect appearing 5 to 15 minutes after intravenous administration. The use of this material in normal animals gave variable results.

11393

Effect of Two Steroid Compounds on Weight of Thymus of Adrenalectomized Rats.*

DWIGHT J. INGLE. (Introduced by F. D. W. Lukens.)

From the George S. Cox Medical Research Institute, University of Pennsylvania,

Philadelphia.

The thymus gland of rats can be made to regress rapidly by the administration of extracts of the adrenal cortex or by the administration of some of the steroid compounds occurring in the extracts. In studies of the biologic effects of 11-desoxy-corticosterone acetate and 17-hydroxy-11-dehydro-corticosterone acetate it was noted that the latter substance was the more active of the two in producing thymus atrophy.

Male rats of the Sprague-Dawley strain each having an initial body-weight of approximately 180 g were used. The diet was Purina Dog Chow. The test substances were dissolved in sesame oil and administered twice daily by subcutaneous injection. Ten normal rats were killed in order to obtain control data on thymus weights; 10 adrenalectomized rats were maintained for 7 days without treatment; 5 adrenalectomized rats were treated with 2 mg daily of 17-hydroxy-11-dehydro-corticosterone acetate; 5 adrenalectomized rats were treated with 2 mg of 11-desoxy-corticosterone; and 5 adrenalectomized rats were treated with 10.0 mg daily of 11-desoxy-corticosterone. Necropsy was performed on the 7th day. The data on body weights and thymus weights are summarized in Table I.

^{*}I wish to express my appreciation to Dr. H. L. Mason of the Mayo Clinic who supplied the sample of 17-hydroxy-11-dehydro-corticosterone acetate; and to Dr. E. Oppenheimer of the Ciba Pharmaceutical Products, Inc., who supplied the 11-desoxy-corticosterone acetate.

		Bo	dy nt	Thy	mus wt
Exper. group	No. animals	Avg	Range	Avg	Range
Normals	10	180	179-151	447.0	355-576
Untreated	10	153.8	143-173	397.0	302-523
2.0 mg daily 17-hydroxy-11- dehydro-corticosterone	5	156.4	148-171	24.6	21-27
2.0 mg daily 11-desoxy- corticosterone acetate	5	200.4	198-203	341.0	285-895
10 mg daily 11-desoxy- corticosterone acetate	5	194.4	186-199	239.0	203-295

TABLE I.

Body-Weights and Thymus Weights of Adrenalectomized Bats.

Although 2 mg daily of 17-hydroxy-11-dehydro-corticosterone acetate did not protect the adrenalectomized rat against a loss in body weight it did produce a marked regression of the thymus. A similar dose of 11-desoxy-corticosterone acetate permitted the adrenalectomized rat to gain in weight during the period of treatment but it did not produce a significant regression of the thymus. The administration of 10 mg daily of 11-desoxy-corticosterone acetate did produce a definite loss in thymus weight but the extent of atrophy was much less than that produced by the 2.0 mg daily dose of 17-hydroxy-11-dehydro-corticosterone acetate. Selye¹ has previously observed a regression of the thymus following the administration of 11-desoxy-corticosterone to rats.

Wells and Kendall² reported that the administration of 11-desoxy-corticosterone acetate to normal rats did not cause regression of the thymus, whereas positive effects were obtained by the administration of corticosterone and its acetate. These workers administered the 11-desoxy-corticosterone acetate in a different manner than was used in this experiment and they did not administer amounts as large as 10.0 mg daily. Ingle and Mason² had previously noted regression in the weight of the thymus following the administration of 17-hydroxy-11-dehydro-corticosterone in solid form to the normal rat. The quantitative relationship of these compounds in respect to the effect upon the thymus is similar to that of their effect upon the capacity of the adrenalectomized rat to work⁴, ⁵ and differs from their relative life maintenance activity.

¹ Selve, H., The Canadian Med. Assn. J., 1940, 42, 113.

² Wells, B. B., and Kendall, E. C., Proc. Stoff Meet, Mayo Clinic, 1940, 15, 133.

³ Ingle, D. J., and Mason, H. L. Proc. Soc. Exp. Biol. and Med., 1938, 39, 154.

⁴ Ingle, D. J., Endocrinology, 1949, 26, 472.

⁵ Ingle, D. J., Endocrinology, in press.

11394 P

Diabetogenic Effect of Some Cortin-Like Compounds.

DWIGHT J. INGLE. (Introduced by F. D. W. Lukens.)

From the George S. Cox Medical Research Institute, University of Pennsylvania,

Philadelphia.

Studies by Long and his coworkers¹ demonstrated the diabetogenic effect of some of the steroid compounds occurring in the adrenal cortex. The effect of 11-desoxy-corticosterone acetate was found to be slight compared to that of corticosterone and 11-dehydro-corticosterone. Jensen and Grattan² have reported that 11-desoxy-corticosterone acetate is much less effective in preventing insulin convulsions in mice than are corticosterone and 11-dehydro-corticosterone.

In the following experiments partially depancreatized³ male rats having a body weight of approximately 280 g were used. The food intake of each animal was kept constant by administering the food

TABLE I.

Effect of Some Cortin-like Compounds on the Glycosuria of Partially Depaneratized Rats.

	TO - 12		Gly	cosuria g	day*
Substance	Daily dose, mg	Rat No.	Before inj.	During inj.	Following inj.
11-desoxy-cortieosterone	1	7	0	0	0
acetate	2	3	0	0	0
	2	3	0	0	0
	2 5 5	3	0	0	0
	5	5	1.75	1.75	1.11
	10	5	1.11	2.12	0.83
	5	8	1.00	3.80	1.78
	10	7	0.95	1.17	0.90
17-hydroxy-11-dehydro-	1	8	1.78	3.70	t
corticosterone acetate	2	2	2.63	4.20	f
	2	3	0	2.12	0
	2	11	0	4.7	Ť
	5	3	0	5.50	0
17-hydroxy-corticosterone	2	7	0.90	5.80	t

^{*}The highest single value for daily excretion occurring prior to injection; the highest value for the response elicited by the injection; and the highest value following the injection period are given here.

†These experiments were terminated by the development of a severe ketonuria. Rats 2, 7, and 11 succumbed. Rat 8 was treated with insulin and recovered.

¹ Long, C. N. H., Katzin, B., and Fry, E., Endocrinology, 1940, 26, 309.

² Jensen, H., and Grattan, J. F., Am. J. Physiol., 1940, 128, 270.

³ Shapiro, R., and Pincus, G., PROC. Soc. EXP. BIOL. AND MED., 1936, 34, 416.

by stomach tube in the manner described by Reinecke, Ball and Samuels.⁴ The test substances were dissolved in sesame oil and administered by subcutaneous injection twice daily. Each series of injections was continued for 4 days. The results are summarized in Table I.

The compound 17-hydroxy-11-dehydro-corticosterone appears to be more potent than 11-desoxy-corticosterone in its diabetogenic effect. In the one test of 17-hydroxy-corticosterone the increase in the glycosuria was very marked, indicating that this substance is also very active. Jensen and Grattan⁵ have observed that the anti-insulin effect of 17-hydroxy-11-dehydro-corticosterone and 17-hydroxy-corticosterone is greater than the anti-insulin effect of 11-desoxy-corticosterone.

The relative effects of the cortin-like compounds on carbohydrate metabolism parallel very closely their effects upon the capacity of adrenalectomized rats to work^o but differ from their relative maintenance activity.

The author is grateful to Miss Dorothy Quinn for technical assistance; Dr. H. L. Mason of the Mayo Clinic, who supplied the sample of 17-hydroxy-11-dehydro-corticosterone acetate and 17-hydroxy-corticosterone; and Dr. E. Schwenk of the Schering Corporation, Bloomfield, New Jersey, who supplied the 11-desoxy-corticosterone acetate.

⁴ Reinecke, R. M., Ball, H. A., and Samuels, L. T., Proc. Soc. Exp. Biol. and Med., 1939, 41, 44.

⁵ Jensen, H., and Grattan, J. F., personal communication.

⁶ Ingle, D. J., Endocrinology, in press.

Alcoholate of Trimer of Hydroxypyruvic Aldehyde as Antidote in Mercuric Chloride Poisoning.

WILLIAM ELLSWORTH EVANS, JR. (Introduced by J. C. Krantz, Jr.)

From the Department of Pharmaeology, School of Medicine, University of
Maryland, Baltimore, Md.

A method for the purification of the alcoholate of the trimer of hydroxypyruvie aldehyde¹ and a study of the metabolism of this compound² have been reported.

The compound depolymerizes readily in aqueous solution, yielding 2 mols of hydroxypyruvie aldehyde and 1 mol of the alcoholate of the monomer and will therefore be referred to as hydroxypyruvie aldehyde. The aqueous solution reduces mercuric ehloride rapidly in the cold, especially in the presence of disodium phosphate.

This investigation was undertaken to determine the capacity of hydroxypyruvie aldehyde and disodium phosphate to aet as an anti-dote in experimental mereurie ehloride poisoning.

Experimental. The experiments were performed on rabbits and eats. All animals were fasted during the 18 hours prior to experimental use, excepting 2 rabbits which were fasted for 48 hours. Mereurie chloride was administered by stomach tube in all of the experiments. Hydroxypyruvie aldehyde and disodium phosphate were given consecutively, whether administered orally or intravenously. Oral doses were followed by a wash of 1 ee of water.

1. Rabbit Experiments. In this series of experiments mercurie chloride was administered in 1% solution, hydroxypyruvie aldehyde in 5% solution and disodium phosphate in 5% solution.

Blood urea-nitrogen levels were determined by Karr's direct Nesslerization method.³ These are recorded in Table II.

The respective groups of rabbits were treated with the antidote orally and intravenously 15, 30, and 60 minutes after the administration of mercuric chloride. These dosages are recorded in Table I. In most cases oral treatment with the antidote was repeated.

2. Cat Experiments. In this series of experiments each cat received 5 mg of morphine sulfate per kg of body weight subcutaneously

¹ Evans, W. E., Jr., Carr, C. J., and Krantz, J. C., Jr., J. A. C. S., 1938, 60, 1628.

² Evans, W. E., Carr, C. J., and Krantz, J. C., Jr., Proc. Soc. Exp. Biol. and Med., 1938, 39, 573.

³ Karr, W. G., J. Lab. Clin. Med., 1924, 9, 3.

TABLE I.
Dosages of Substances Administered to Rabbits.

	Dos	age (mg/kg)
Substance	Oral	Intravenous
Mercuric Chloride	20	0
Hydroxypyruvic Aldehyde	500	125
Disodium Phosphate	250	65

one hour before the experimental poisoning in order to prevent vomiting. Mercuric chloride was administered in 2% solution and hydroxypyruvic aldehyde and disodium phosphate in 10% solution.

Groups of cats were treated with hydroxypyruvic aldehyde and disodium phosphate at intervals of 1, 5 and 15 minutes respectively, after the administration of a fatal dose of mercuric chloride. The results are shown in Table III.

Discussion. Autopsies performed on rabbits No. 10, No. 21, and No. 24 showed no gross pathology of the gastrointestinal tract or kidneys. On examination of rabbit No. 10 the liver was found to be highly parasitized and there were hemorrhagic patches on the lungs. Death of rabbits No. 23 and 25 on the eleventh day of the experiments may have been caused by unusually hot weather. This conclusion is supported by the blood-urea-nitrogen determinations made on the ninth day. These determinations indicated that there was slight or no renal impairment at this time.

Treatment of 11 rabbits with hydroxypyruvic aldehyde and disodium phosphate one hour after the administration of mercuric chloride prevented renal injury in 9 animals. Decreased chance of survival after prolonged fasting is indicated by the blood urea-nitrogen levels of the 2 animals which had been fasted for 48 hours prior to experimental poisoning. Oral treatment with the antidote again on the second day of the experiment may favor recovery.

Oral treatment of 11 cats with hydroxypyruvic aldehyde and disodium phosphate one minute after the administration of mercuric chloride prevented acute poisoning. Nine animals survived longer than 30 days. The other 2 cats died on the seventeenth and twenty-fourth days, respectively, of the experiment. The antidote did not protect cats effectively if 5 minutes had elapsed before treatment but did increase the average survival time. Hydroxypyruvic aldehyde and disodium phosphate had no antidotal action when administered 15 minutes after mercuric chloride had been given.

Conclusions. Hydroxypyruvic aldehyde in the presence of disodium phosphate acted, within the limits set forth in these experiments, as an effective antidote against mercuric chloride poisoning in rabbits and cats.

TABLE II. Blood Urea-Nitrogen Determined on Rabbits Poisoned with Moreurie Chloride and Subsequently Treated with Hydroxypyruvic Aldehydo

					เทส มารอ	արտ բո	and Disodulin Phosphate.					
, o	Time bet.					Blood a	Blood urea-nitrogen (mg%)	gen (m)	(%5			
rabbits in	poison and	Rabbit	Survival				Day of	Day of experiment	'nt		[
group	min.		days	Centrol	6.1	۳	-#	12	9	l-	6	Remarks
က		9 1	ကင	16.8	1	218	534					
		· œ	3 es	15.9	1	276						
က	15	21 21 21 21 21 21 21 21 21 21 21 21 21 2	>30	17.4 7.6	1	14.3	17.6	1	910			Only phosphate adm.
		7	-11	ro c i	1	172	237					ed 2nd and 3rd days
က	15	e č	°,30	21.8	1	19.0	14.8	!	18.5			
		21	30°*	7.7		32.1 18.6	6.45 6.45 6.45 6.45 6.45 6.45 6.45 6.45	1	14.6			Ornl antidote repeat-
က	30	10	/30	0 01	1				0.1.1			ed End and 3rd days
	2	10) 14.0	1 6 1 6	35.0 0.05 0.05		1	15.3				
		17	23	18.8	33.2	16.3	1 1	30.4				Oral antidoto repeat-
		18	13	24.1	23.0		100	!	į			ed zna any
		10	% ∧	20.3	96		001/	1	2,1	,		
		50	≥ 30	13.2	50°		13.0	1	1	:: :::::::::::::::::::::::::::::::::::		Oral antidoto repeat-
		ឥខ	* \ \	11.4	20.6		13.6	1 1	1 1	#::- 10 01		ed 2nd day
F	;	ì	730	18,9	20:5		14.8	ł	}	19.4		
7	09	63 5	11	19.7	35.2	23.5	}	i	}	1	0	
		47 E	: : : : :	16.7	38.0	47.5	1	27.3	j	1 1	0 to	
		9 9 9	1 ~	11.3	6.1 6.3 6.3	1	1	15.9	1		14.4	
		33	>30	19.2	1	I	102		c E			
		34	>30	17.2	}	1	32	11	13.0	11	66.6 5.9 8.9	Fasted 48 lirs
> Mo	> More than,											ed after 5 hrs

* Sacrificed for autones

TABLE III.
Antidote After Oral Administration of Mercuric Chloride te Cats.

		Time interval	7	Antidote (mg por kg)	(mg bc	r kg)				
Cats in group	Dose ef mercuric chloride,	bet, adm. of poison and antidete,	Hydroxypyruvic aldehyde	lroxypyruvic aldehyde		Disodium phosphato	Disodium phosphato	Surviv	Survival peried	Avg survival peried
Ne.	By/Bu	min	Oral	I.V.		Oral	I.V.	No.	Days	in tatal cases, days
m	25		0	0		0	0	H 63	01	1.7
က	0		250	62		125	31	က	^ 30	
ဗ	25	ເຈ	1320	62		125	31	п пппп	H 83 44 80	89. 52.
ເວ	25	15	250	62		125	31	HOH	H 23 ES	2.0
13	25	15	200	0		250	0	7040	7 30 7	65,63
4	25	ro.	200	0		250	0	ฌ๚๚	10 14 14	7.0
11	25	Ħ	200	0		250	0	H H G	17 24 \	20.5

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Alterations in the Blood Histamine in Shock.

Bram Rose and J. S. L. Browne.

From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Within the last decade much experimental data has accumulated on the subject of shock, and the explanation of its mechanism has been greatly elarified. There is still considerable controversy, however, about the actual factors responsible for the production of this syndrome. A discussion of the prevalent theories cannot be undertaken here for reasons of space but excellent reviews are available. (Moon.¹)

In an effort to investigate the relationship of histamine to various elinical conditions, the total blood histamine of a series of normal individuals was determined, using the method of Barsoum and Gaddum² as modified by Code.³ Since the production of reactive hyperemia by prolonged stasis of the circulation has been demonstrated to increase the blood histamine (Barsoum and Smirk'), specimens were obtained observing precautions against stasis. In a series of 50 controls the average figure was found to be 0.04 y/cc (as base) with variation of from 0.025 to 0.08 y/cc. These figures agree in general with those of Haworth and MacDonald,5 although they found a variation from 0.018 y/ec to 0.078 y/cc. In repeated examinations of the blood of the same individual from time to time over a period of months, it has been found that the blood histamine remains at a fairly constant level. For example, in a patient whose first sample contained 0.07 y/cc subsequent samples at intervals of 2 days contained 0.07, 0.065 and 0.07 y/cc. Three more at weekly intervals and a later one taken 2 months after the initial examination all gave values of 0.07 y/cc. In several of the patients, one with an initial blood histamine value of 0.05 y/ce and another with 0.04 y/cc similar results were obtained. In a series of 150 patients with various conditions, 19 cases presenting shock varying from a mild form to severe collapse as determined by clinical signs and blood

¹ Moon, V. H., Ann. Int. Med., 1938, 12, 205; Shock and Related Capillary Phenomena, Oxford Univ. Press, 1938.

² Barsoum, G. S., and Gaddum, J. H., J. Phys., 1935, 85, 1; Clin. Sci., 1936, 2, 357.

³ Code, C. F., J. Phys., 1937, 89, 257.

⁴ Barsoum, G. S., and Smirk, F. H., Clin. Sci., 1935, 2, 353.

⁵ Haworth, E., and MacDonald, A. D., J. Hyg., 1937, 37, 234.

studies including plasma specific gravity, hemoglobin and hematocrit estimations were studied. In 4 cases, a single determination only was obtained. Control specimens were taken either before operation or after recovery in the remaining 15. These have been divided into 3 groups. Table I gives the histamine values obtained on 8 patients who underwent surgical operation without manifesting any clinical or other signs of shock. One case of severe trauma in a child of 8 is also included.

It will be observed that little or no change in the blood histamine occurred in 5 of these patients. In 2, however, (No. 155 and 162) a moderate decrease occurred. Blood studies were performed simultaneously and in no instance was there any degree of hemoconcentration. One exception in this group is patient No. 96 in whom a single determination only was obtained several hours after operation and the value for blood histamine was $0.015 \, \gamma/cc$, which is low as compared to the normal average. He did not have any symptoms of shock.

TABLE I.

Blood Histamine in Patients Undergoing Surgical Operation Without Developing Shock.

Case No.	Age	Sex	Operation	Time of specimen	Blood histamine
70	30	F	D and C	Control 1 hr	.05 .03
96	32	M	Nephrectomy	4 hr	.015
117	30	M	Gastric resection	Control 4 hr	.06 .05
134	34	M	Gastric resection	Control 1 hr 2 hr 24 hr	.065 .075 .05 .05
148	45	M	Gastric resection	Control 3 hr	.035 .042
155	36	F	Thoracotomy	Control 3:30 hr 24 hr 7 days 14 days	.062 .035 .033 .025 .042
162	50	F	Cholecystectomy	Control 2 hr 24 hr 2 days 8 days	.06 .05 .04 .06
93	9	M	Trauma to leg followed by amputation	1 hr after trauma 1 hr after amputation	.04

		11.	'rablus al recheming developing Shork Pollowing Surgical Interference.	inek Following Surgie	al Interference.	
) 0	Bilood His	Operation	Timo specimen taken	Signa of shock	Blood histamine $\gamma/$ ec
Case No.	M	35	Gnstrie resection	4 hr P.O. 3 days P.O.	+++ Recovered	.01
೮೦	ž	21	Bilateral hydronephrosis	6 days P.O.	+++	60 °
ස ස	N	다	Gastric resection	12 hr P.O. 6 days P.O.	+++ Recovered	.01 00.
102	Х	65	lst stage prostatectomy	5 hr P.O. 48 hr P.O. 7 days P.O.	++++ Recovered	.015 .08 .08
130	ĸ	45	Abdomino-perineal resection	Control 134 hr P.O. 24 " P.O.	++ Condition good	.035 .01 .04
141	×	48	Aeuto intestinal obstruction, patient operated on	24 hr 2 '' P.O. 34 '' P.O. 48 '' P.O. 73 '' P.O.	+ + +++5 ++++5	.01 .06 .02.5 .01
161	ᄄ		Gastroduodenostomy	Control 3 hr P.O. 5 '' P.O. 24 '' 48 '' 8 days	++ ++	.03 .03 .03 .03 .038
116	¥	31	Pyloroplasty	Control 24 hr P.O. 48 '' 7 days	+ +++	0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.

TABLE III. Cases of Traumatic Shock.

Case No.	Sex	Age	Trauma	Time specimen taken	Signs of shoot	Blood histamine
-				Transa transaction and the	Cigus or succe	33/4
41	M	28	Industrial accident, hit by falling bricks	4 hrs after	++++	200*
118	M	50	Severe injury to right arm, with hemorrhade	1 hr after trauma	++++	.11
			Operation amputation of arm	4 hr P.O. 18 '' P.O. 20 ''	G++	.02
131	ᄄ		Head injury with hemorrhage		+ + +	.015

Eight patients who exhibited signs of shock following operation are presented in Table II. Six of these showed marked changes in the blood histamine, and this was also accompanied by evidences of hemoconcentration as determined by simultaneous blood studies. It will be observed that as a general rule, the blood histamine is low or decreasing within 2 to 3 hours following the operation and that there is a return to normal or even high levels after varying intervals of time.

In Table III are presented 3 patients admitted following severe trauma. Single determinations only were available on 2 of these, No. 4 and 131, and it will be noted that the blood histamine level is decreased. Case 118 was admitted to the hospital within 45 minutes after having sustained a severe traumatic injury complicated by hemorrhage. A specimen was obtained before any therapy was administered and the blood histamine was found to be $0.11 \, \text{p/cc}$. This is higher than the normal value. Following amputation of the right arm, and administration of cortin intravenously along with a transfusion, the blood histamine was again determined and found to be $0.04 \, \text{p/cc}$. On the following day, a third specimen was found to be $0.02 \, \text{p/cc}$ at $10.30 \, \text{A.M.}$ and at $12.30 \, \text{P.M.}$ the patient died. It should be noted that in all other cases, the first examination of the blood was done only several hours after the trauma.

A single determination only was obtained in 6 cases in whom death occurred within 3 to 48 hours. These are presented in Table IV and it will be noted that the blood histamine is markedly decreased in all as compared to normal values.

The histamine theory of shock has been rejected by many different

TABLE IV. Cases in Agonal States.

Case No.	Sex	Age	Diagnosis	Time of death after T operation or onset of symptoms	ime before death specimen taken	Histamine
86	F	45	Severe burns	7 days	24 hr	.001±
111	F	33	Dehydration colitis		24 "	.015
119	M	40	Mescuterie thrombosis	36 hr	12 ''	.015
123	\mathbf{F}	35	Extreme eachexia	6 mo	48 ''	.001
135	M	60	Ca of stomach		48 ''	.01
167	F	30	Pneumonia and peritonitis following appendectomy	7 days P.O.	3 "	.005

workers mainly because of the difficulty of demonstrating the presence of an active depressor substance in the blood of experimental animals or in that of man during shock (Schneider, 6 O'Shaughnessy and Slome, Dragstedt and Mead8). On the other hand, histamine is looked upon as a factor responsible for the production of symptoms of anaphylactic shock in the guinea pig (Bartosch, Feldberg and Nagel, Code¹⁰), and in the dog (Dragstedt and Mead, Code¹⁰). In both of these species, anaphylactic shock is accompanied by an increase of the blood histamine. Furthermore, there is little difference between the symptoms of anaphylactic shock and histamine shock in either of these species or in the rabbit. Yet, in the rabbit, the onset of anaphylactic shock is accompanied by a marked decrease in the blood histamine (Rose and Weil¹²). This has also been shown to occur in the horse and calf (Code and Hester¹³). It may be of interest to note that according to Moon¹ there is a fundamental similarity in the pathology of anaphylaxis and shock, namely changes in capillary function and endothelium.

Previous reports on the blood histamine in cases of shock due to extensive burns have indicated that there is first an increase of blood histamine followed by a slow return to normal (Barsoum and Gaddum, Code and MacDonald.). According to Barsoum and Gaddum there was no relation between this rise and secondary shock. Only one case with severe burns has been available in the present study, in this a simultaneous study of the blood histamine, hemoconcentration and blood electrolytes was made. (Case No. 86). The blood showed marked hemoconcentration, and no histamine was found. This specimen was obtained on the sixth day after the trauma and the patient died 48 hours later.

In view of the above results, there appears to be a mechanism whereby the blood histamine is diminished in conditions where the accepted criteria of shock exist, and in certain other cases shortly before death. The nature of this mechanism is not yet clear, but may possibly be due to a transfer of the histamine of the blood to the

⁶ Schneider, H., Deutsche Z. f. Chir., 1930, 229, 343.

⁷ O'Shaughnessy, H. L., and Slome, D., Brit. J. Surg., 1935, 22, 589.

⁸ Dragstedt, C. A., and Mead, F. B., J. Am. Med. Assn., 1937, 108, 95.

⁹ Bartosch, R., Feldberg, W., and Nagel, E., Arch. f. d. ges. Physiol., 1932, 230, 129.

¹⁰ Code, C. F., Am. J. Physiol., 1939, 127, 78.

¹¹ Dragstedt, C. A., and Mead, F. B., J. Pharm. and Exp. Ther., 1936, 57, 419.

¹² Rose, B., and Weil, P., PROC. Soc. EXP. BIOL. AND MED., 1939, 42, 494.

¹³ Code, C. F., and Hester, H. R., Am. J. Physiol., 1939, 127, 71.

¹⁴ Code, C. F., and MacDonald, A. D., Lancet, 1937, 233, 730.

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119	M	40	Mesenteric thrombosis	36 hr	12 ''	.015
123	F	35	Extreme enchexia	6 mo	48 ''	.001
135	M	60	Ca of stomach		48 ''	.01
167	F	30	Pneumonia and peritoaitis following appendectomy	7 days P.O.	3 "	.005

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Extracts of Anterior Pituitary Growth Hormone.

AMOS E. LIGHT, EDWIN J. DEBEER AND CHARLES A. COOK. (Introduced by L. Reiner.)

From the Burroughs Wellcome & Co. U.S. A. Experimental Research Laboratories, Tuckahoe, V. V.

The solvent action of phosphate buffer, urea and sodium hydroxide solutions in extracting the growth principle of the anterior pituitary gland was investigated with the aid of a comparatively simple bioassay method. The potency of each extract was determined and expressed as mg per cc of a standard anterior lobe powder. The percent yield was calculated by dividing the potency by the number of mg of powder used per cc in making the extract.*

To facilitate comparison, all extracts were prepared in as uniform a manner as possible from the same desiccated, powdered anterior lobe substance? which also served as the standard for all assays. For example, phosphate buffer extract No. 1 (Table I) was prepared by extracting 25 g of powder with 200 cc of a solution (pH 8) containing 5.616 g of anhydrous Na₂HPO₄ and 0.302 g of anhydrous KH₂PO₄ per liter. After 3 hours of continuous stirring at 10°C, the insoluble residue was separated by centrifuging and successively extracted with three 125 cc volumes of buffer solution. The combined extract, which had a pH of 7.5, was filtered through cellulose and asbestos pads, sterilized by filtration through a Seitz apparatus and stored at 10°C in sterile rubber-capped bottles. After

^{*}Inspection indicates that the dose-response curve (Fig. 1) for the extracts resembles that for the powder suspension. An experiment sufficiently elaborate to establish the linearity of the relationship between log dose and response will be reserved for a selected extract since it is obviously impractical to do this for each extract. However, at least 2 and often 4 graded doses of each extract were given (intraperitoneally) thus establishing the slope for each extract curve. The standard was employed in each assay. In the following example, the standard contains 5 mg of powder per co.

Star	dard	Extra	ect &D
Dose	Bespense	Dose	Besponse
cc/100 g rat	% gain in wi	cc/100 g rat	रत हार्या में कर
0.05	1.3	0.01	2.0
0.10	3.6	0.02	3.4

Therefore, I ee of extract containing about 10 mg of protein is equivalent to 6.4 ee or 32 mg of standard powder.

¹ Light, A. E., deBeer, E. J., and Cook C. A. in press.

i Burroughs Welleome & Co. (The Welleome Foundation, Ltd.), London.

damaged area or to certain of the abdominal viscera. This may possibly account for the failure to demonstrate an increase in the histamine content of the blood when shock is well established, although some of the cases indicate that there may be an early increase. may be that there is first a liberation of histamine which is rapidly followed by a decrease of the blood histamine below the normal value. This could be accounted for if one assumed that the histamine gathers in the tissues, or traumatized area, as it does in areas of inflammation (Tarras-Walilberg,18 Rocha e Silva and Bier16). Furthermore, in dogs it has been shown that following burns there is first an increase of the blood histamine, followed by a disappearance in a matter of 2-3 days, accompanied by an increase in the histamine content of the spleen, liver and pancreas (Kisima¹⁷). There is a marked similarity between adrenal insufficiency and shock and it has been demonstrated that the histamine content of the gastro-intestinal tract increases to 250% over the normal in adrenalectonized rats, although there is little change in the blood histamine of these animals.

It should be pointed out that although the blood histamine is markedly diminished in patients where shock is established, such a diminution may occur in certain other conditions in cases of allergy. In these latter states, however, one does not observe as marked a variation in the histamine content of the blood. This will be discussed in a subsequent communication.

Conclusions. In a series of patients in varying types of shock manifested by clinical signs, hemoconcentration, and lowering of the B.P. the total blood histamine has been determined. The results indicate that in the cases studied a marked diminution of the histamine content of the blood occurs when shock is established as compared with control values and those after recovery. There appears to be some correlation between the severity of the shock and the degree of blood histamine decrease. Blood histamine has also been found to be low in agonal states. The significance of these findings is discussed.

¹⁵ Tarras-Wahlberg, B., Klin. Wschr., 1937, 16, 958.

¹⁶ Rocha e Silva, M., and Bier, O., Arq. do Inct. Biology, 1938, 9, 123.

¹⁷ Kisima, H., Fukuoka Acta. Medica, 1938, 31, 49.

several weeks a precipitate appeared in this extract. The high yield indicates that most of the active material was extracted. About 1% of the extract was organic matter, probably protein. This figure was approximated by 3 different methods, *i.e.*, by trichloracetic acid precipitation, by calculating the total N as protein and by subtracting the ash, which was largely due to buffer salts, from the total solids.

In contrast to the buffer extracts, the gelatinous nature of the extracts prepared with dilute aqueous NaOH solutions made them very difficult to clarify by filtration. Furthermore, a considerable precipitate formed after one week of storage. In preparing this type of extract, NaOH was added at intervals in order to maintain the pH at 7.5. These extracts were not as uniform in potency as those obtained with phosphate buffers.

Urea, in high concentrations has been shown to bring about remarkable changes in the chemical and physical properties of certain proteins.^{2, 3} Accordingly, a study was made of the properties of phosphate buffer extracts containing 1%, 5%, 10%, and 20% urea. (Table I.) These preparations were of about the same potency as the phosphate buffer extracts. The addition of urea in concentrations as low as 1% or 5% retarded precipitation for several months, particularly if the urea were added just before filtration through a Seitz apparatus. When the concentration of urea was 10% or 20%,

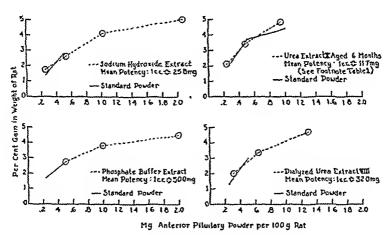


Fig. 1.

Dose response curves for anterior pituitary extracts in terms of standard powder and compared with standard powder curves.

² Steinhardt, J., J. Biol. Chem., 1938, 123, 543.

³ Greenstein, J. P., Ibid., 1939, 128, 233.

TABLE I. Extracts of Anterior Pituitary Growth Hormone.

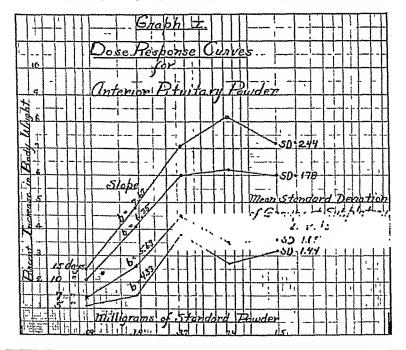
				Limits	Limits of error				
		7,000	7,000	as % 0	as % of mean	Protein			
No.	Preparation	poteney* mg/ec	Yield %	Lower %	Upper	$\begin{array}{c} {\rm Triehlor}, \\ {\rm acctic\ neid} \\ {\rm v}_{o} \end{array}$	Total solids ?3	Λ ₈ h	Nitrogen (Kjeldahl)
,	Desiceated anterior lobo (standard)						88.0	5.0	11.5
H 63 6	Phosphate buffer Phosphate buffer	50,0 39,2	100	58	173	1.18	1.57	0.52	0.13
ত বা	NaOH 1% Urea	95.8 -	63	ទ	165	1.47	08.1 1.43	0.70	0.14
ro c	5% ''	20.0	7 0 7	<u> </u>	175 175	1.00		•	× • • • • • • • • • • • • • • • • • • •
a	10%	20°0 30°0 30°8	100	និ	168	0.99			
7 8D	20% ''	30.5	16	35	189 156	0.99 1.15			
e 6	10% " (pii 9)	35.0	35	6 99	145	111	1.63	0.54	0.321
10	10%	80° C	68	75	134	1.12			1140
=	1% Gunnidine	20.3	<u>:</u>	8 g	173	1.00			110%
*	Moon notes.								

* Mean potency determined in terms of mg of standard powder per ee of extract, 4 0.28% urea still present. 4 Double volume. Dialyzed to remove urea.

growth hormone preparations. Groups of 15 normal, plateaued, female rats, approximately 5 months of age, were selected with respect to strain (Yale), weight (240-300 g) and diet (Fox Chow). Three preliminary weighings during a 5-day period were necessary to establish the fact that a plateau level in the normal growth curve, essential for the assay method, had been reached. To accustom the animals to the standardized routine treatment, injections were also made at these times with saline, phosphate buffer (pH 8) or 10% urea in 0.04 molar buffer solution.

Well-defined dose-response curves were obtained when daily, graded doses of a saline suspension of desiccated anterior lobe substance* were injected intraperitoneally (Graph I). A sufficient quantity of this material was also available to serve as a standard for subsequent assays. Statistical analysis of a curve based on results obtained with 165 rats revealed that on the steeply ascending portion of the curve, below the plateau level, the per cent increase in body weight was a linear function of the logarithm of the dose.

In routine assays, 2 groups were injected with sub-plateau doses



^{*} Burroughs Wellcome & Co. (The Wellcome Foundation, Ltd.), London.

the extracts were still clear at the end of 6 months. When extracts 6 and 9 were dialyzed through cellophane membranes about 95% of the urea was removed and precipitates appeared in the corresponding preparations, 6D and 9D, in about 3 weeks.

As extracts 9 and 10 indicate, it appeared unnecessary to increase either pH or volume in order to improve the efficiency of the extraction. The 1% guanidine extract yielded an amount of hormone similar to that of the 1% urea.

Summary. Phosphate buffer extracts of growth hormone were highly active when assayed in terms of anterior pituitary powder. This method of extraction permitted a careful control of pH and gave high yields of hormone. These extracts had less tendency to form precipitates than those prepared with sodium hydroxide. The addition of urea retarded such precipitation.

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Biological Assay of Anterior Pituitary Growth Hormone.

Amos E. Light, Edwin J. deBeer and Charles A. Cook. (Introduced by L. Reiner.)

From the Burroughs Wellcome & Co. U. S. A. Experimental Research Laboratories, Tuckahoe, N. Y.

Normal animals have been employed in the assay of growth hormone by Evans et al., Van Dyke and coworkers and Lee. Inherent limitations of such an assay method have emphasized the importance of statistical treatment of the data. Bülbring, working with hypophysectomized rats, has utilized the rapidly rising portion of a doseresponse curve and has reported results which indicated relatively low limits of error.

In order to avoid the complex metabolic derangements associated with an extirpation of the entire pituitary gland, an assay procedure was developed in which groups of normal rats were used to determine the increased body weight resulting from administration of

¹ Evans, H. M., Uyei, N., Bartz, Q. R., and Simpson, M. E., Endocrinology, 1938, 22, 483.

² Chou, C., Chang, C., Chen, G., and Van Dyke, H. B., Ibid., 322.

³ The Pituitary Gland, Proc. Assn. for Research in Nervous and Mental Disease, 17. Williams & Wilkins, Baltimore, 1938, 216.

⁴ Bülbring, E., Quart. J. Pharm. and Pharmacol., 1938, 11, 26.

potency should fall 21 out of 22 times, can be obtained by dividing the potency by the antilog of 2 S.E. for the lower limit and multiplying the potency by the antilog of 2 S.E. for the upper limit.

As shown in Graph I, a longer period of treatment results in an increased slope of the sub-plateau portion of the curve. This would tend to narrow the limits of error but it is partly offset by an increase in the standard deviation. The limits of error when calculated as described above were 67-149, 70-142, 71-140 and 68-147% of the mean potency for the 5-, 7-, 10- and 15-day periods of treatment. respectively. These limits of error have been confirmed by additional assays with normal rats. The average of 31 standard deviations was 1.6 for the 5-day assay. In view of these results, the 5-day period was used for routine assays. Bülbring's data from hypophysectomized rats gave limits of 49-206% for the 7-day period when calculated as above. These wider limits are largely due to the fact that Bülbring used only 5 animals per group instead of 15 as used in the present study, since the ratio $\frac{S.D.}{b}$ is approximately the same for both sets of data. Bülbring's ratio is $\frac{4.95}{14}$ or 0.354. The corresponding figures from Graph I are $\frac{1.65}{5.63}$ or 0.293. The actual values for standard deviation and slope are not comparable since Bülbring expresses the response in grams, while in Graph I response is given in per cent.

In comparing 2 preparations assayed against the same standard, but at different times, the standard error, by the formula for the standard error of the difference of 2 means is

$$S.E._{(M_1 - M_2)} = \sqrt{(S.E._{M_1})^2 + (S.E._{M_2})^2}$$

is the s.e. $_{M_1}$ is the standard error of one assay and s.e. $_{M_2}$ where standard error of the other assay. Since these values are approximately equal, the standard error is the $\sqrt{2}$ or 1.41 times the standard error of a single assay, which for a 5-day period gives limits of about 57-174%. This means that for 2 preparations, each assayed against the same standard powder, one must be nearly twice as potent as the other in order to be considered significantly different. Evidence is being accumulated as to the stability of the hormone in the anterior pituitary powders and in experimental extracts.

⁶ Coward, K. H., Biological Standardisation of the Vitamins, Balliere, Tindall and Cox, London, 1938, 166.

of the standard powder* and two with the experimental extract. For each preparation the ratio of the greater dose to the smaller should be at least 2 to 1. The standard powder was suspended in a saline, buffer or urea solution and the assay data from 5 daily injections were used to calculate the potency of experimental preparations in terms of milligrams of the standard powder. Final group weights were recorded 120 hours after the initial injections of either the standard powder or the extracts. The groups were also weighed daily at the time of injection for evidence of any irregularity in body weight. Control groups injected with saline, 10% buffer solution or various doses of an inactive pituitary extract showed no significant changes in body weight.

Gaddum⁵ has shown that the statistical evaluation of biossays involving a linear relationship and the use of a standard substance can be greatly simplified by following the plan given below. Using 4 groups, each containing an equal number of animals, let X_{11} , X_{12} be 2 doses of the standard preparation and X_{21} , X_{22} the doses of the test preparation, and the corresponding mean responses of each group be Y_{11} , Y_{12} , Y_{21} and Y_{22} . The responses were expressed as per cent gain in body weight. Let d, the log of the ratio of the greater to the smaller dose, i. c., $\frac{X_{22}}{X_{21}}$, be the same for each preparation. The following equation will then express the potency of the test preparation in terms of cc of the standard solution.

1 cc of test solution =
$$\frac{X_{11}}{X_{21}}$$
 antilog $\left(\frac{Y_{21} + Y_{22}}{2b} - \frac{Y_{11} + Y_{12}}{2b}\right)$

The term b, or slope, serves to convert each average response, i. e., $\frac{Y_{21} + Y_{22}}{2}$ and $\frac{Y_{11} + Y_{12}}{2}$, into the logarithms of the corresponding average doses. Since the resulting terms are logarithmic, the difference between the two values represents the antilog of the ratio of the two average doses. The average slope, b, is given by the expression,

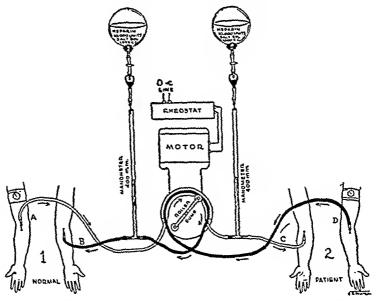
$$b = \frac{1}{2} \left(\frac{Y_{22} - Y_{21}}{d} + \frac{Y_{12} - Y_{11}}{d} \right).$$

The standard error of the assay, expressed logarithmically, is S.E. $=\frac{\text{S.D.}}{b}\frac{1}{\sqrt{n}}$, where S.D. is the standard deviation, b is the slope and n is the number of animals in a single group. When restricted to the sub-plateau levels, the standard deviation was independent of the size of the dose. The limits of error within which the calculated

⁵ Gaddum, J. H., Med. Res. Council, Special Report Series No. 183. 1933, 31.

two, a uremic patient and a normal donor, were then connected by a suitable rubber tubing filled with heparin in saline. The tubes passed through a roller pump which milked forward the same quantity of blood in opposite directions. A 400 mm manometer was placed between the pump and the recipient (1B and 2C), thus providing a sensitive indicator of any obstruction in the needle and at the same time a safeguard against undue increasing pressure in the receiving vein (Fig. 1).

Sufficient bicarbonate of soda was given to the donor and the recipient to make the urine alkaline as a preparatory measure. Trials of the apparatus in artificial models gave good results. Two patients with chronic uremia, one as a result of a polycystic disease of the kidneys and the other from chronic glomerular nephritis, have been given continuous reciprocal transfusions from normal donors. In the first patient an exchange of 2,520 cc of blood (including saline and heparin) was made over a period of 2 hours and 50 minutes. On the second patient 7,020 cc of blood, not including saline and heparin, were cross transfused in 47 minutes; during the greater portion of the time 82 cc of blood were delivered in each direction per



Pig. 1.

Diagram of the apparatus. 1A—delivering vein, 1B—receiving vein of the normal individual. 2D—delivering vein, 2C—receiving vein of the uremic patient. The 2 tubes rest exactly superimposed in a semi-circle around the periphery of the roller pump.

Summary. A selected anterior lobe powder from ox pituitary was used successfully as a standard of reference for one year in an assay procedure employing normal rats. By restricting the biological comparison to the steeper portion of the dose-response curves it was possible to obtain relatively low limits of error.

11399 P

Application in Man of Method for Continuous Reciprocal Transfusion of Blood.*;

GARFIELD G. DUNCAN, LEANDRO TOCANTINS and TRACY D. CUTTLE.

From the Medical Service of the Pennsylvania Mospital, and the Department of
Medicine, Jesseson Medical College, Philadelphia.

Purified heparin, by prolonging the coagulation time of blood, has reduced the technical difficulties and made feasible the continuous reciprocal transfusion of blood in man. Thalhimer, Solandt and Best, using a uremic and a normal dog and employing purified heparin as an anticoagulant, conducted a reciprocal transfusion for 27 hours, reducing the blood urea from high levels to normal without injury to the normal dog. Prinzmetal carried out exchange transfusions in the investigation of arterial hypertension in patients with inoperable cancer. We have been unable however, to find any instance in which the work of Thalhimer et al. was applied in man.

Heparin (10,000 Toronto units in 1,000 cc normal saline) was given intravenously at approximately the rate of 40 drops per minute to the normal donor and to the patient for 20 minutes before and throughout the transfusion. An additional 2,000 units were given intravenously to each individual as the transfusion was started, and repeated 30 minutes later. This maintained the blood coagulation time between 20 and 30 minutes. The median basilic veins of the

^{*} The authors are indebted to Dr. William A. Wolff, Chemist to the Pennsylvania Hospital, for valuable suggestions on the chemical aspects of this problem.

[†] Since this paper was submitted for publication a transfusion was carried out in which 26,770 ee were exchanged in approximately 5 hours. The total nitrogen exerction in the urine increased from 548 mg per hour before to 851 mg per hour during the transfusion.

¹ Thalhimer, W., Soldant, D. G., and Best, C. H., Lancet, 1938, 2, 554.

² Prinzmetal, M., Friedman, B., and Rosenthal, N., Proc. Soc. Exp. Biol. AND Med., 1936, 34, 545.

11400

The Rôle of Boron in the Diet of the Rat.*

ELSA ORENT-KEILES.

From the Laboratory of Biochemistry, School of Hygiene and Public Health, The Johns Hopkins University.

Boron is of practically universal occurrence in the plant kingdom. Hence it is apt to be present in almost all classes of foodstuffs, and therefore occurrence of this element might be equally widespread in the animal kingdom and perhaps might play an equally important rôle in the physiology of the animal organism. At present little is known regarding the action or even the distribution of boron in animals. There are but few reports on the distribution of this element in animal tissues.¹⁻⁴ These few briefly-reported experiments indicate the necessity for further knowledge of the rôle of boron in the animal organism. As far as was known at the time this investigation was started, no data were on record as to whether boron is a dietary essential for animals. It seemed advisable, therefore, to determine whether boron is required for the growth and development of the rat or whether it is present in the animal body as an accidental constituent ingested with all foods.

Experimental. Experience with the study of other trace elements has shown that to do so effectively, a diet extremely deficient in the element must be used. The natural foods commonly used in purified rations were too abundant in boron. Cow's milk, although lower in this element, is not as low as is desirable and furthermore, a dry ration is preferable for such studies. Hence an attempt was made to produce such a diet depleted of its boron content. Naftel's micro method, using a photoelectric colorimeter, was employed for the determination of boron in the various foods studied. The ashing procedure was somewhat modified by using the overhead heater which has proven advantageous in the matter of time saved and in the prevention of loss of boron due to excessive smoking and swelling

^{*} This work was aided by a grant from the Rockefeller Foundation.

¹ Bertrand, G., and Aguihon, H., Compt. rend., 1912, 155, 248; 1913, 156, 732, 2027.

² Wright, N. C., and Papisch, J., Science, 1929, 69, 78.

³ Blumberg, H., and Rask, O. S., J. Nutr., 1933, 6, 285.

⁴ Drea, W. F., J. Nutr., 1934, 8, 229; 10, 351; 16, 325.

³ Naftel, J. A., Ind. and Eng. Chem. Anal. Ed., 1939, 11, 407.

minute. In neither study were there any untoward reactions, except a slight elevation of temperature in one of the normal donors, attributed to a large hematoma having occurred when the needle escaped from the receiving vein.

Clinically both patients seemed improved. In Table I are listed the average hourly excretion of nitrogen in the urine of the two patients and the normal persons, as an example of the metabolic changes induced by the reciprocal transfusions. Further observations by this method, now under way, may help to elucidate obscure points in various metabolic disorders and perhaps make it possible to afford some relief during acute, though transient, incapacitations of the urinary apparatus.

TABLE I.

Total Nitrogen Exerction (mg per hr) in the Urine of the 2 Uremic Patients and
the 2 Normal Donors Before, During and After the Reciprocal Transfusion.

		Nitrogen Excretion (mg per hr)							
		Jremie Pa	tient (м.в.)	2	Normal Donor (A.P.)			
Period	Fotal N	Urea N	Uric Acid	Creatinine	Total N	Urea N	Urie Acid	Creatinine	
12 hrs prior to transfusion 4% hr including transfusion	230	190	5.6	13.5	306	232	3.9	21.4	
period Subsequent 6 hr	322 301	229 246	$\frac{5.6}{4.6}$	16.8 18.8	471 342	428 316	15.0 8.0	32.5 30.0	
6-18 hr after transfusion	253	227 Uremic Pı		15.1	399	356	Donor (31.4	
13 hr 55 min prio		Oremie 1-1	ment ((15.0.)	1	01111111	Donor ((1).0.7	
to transfusion 334 hr including transfusion		262	2.6	13.5	398	340	1.0	16.5	
period	478	339	4.3	16.3	486	412	6.3		
6 hr following this period Subsequent	191	118	1.3	10.0	302	173	6.3	30.0	
6-1936 hr period	262	228	1.9	_	378	239	4.6	20.0	

11400

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¹ Bertrand, G., and Agulhon, H., Compt. rend., 1912, 155, 248; 1913, 156, 732, 2027.

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⁴ Drea, W. F., J. Nutr., 1934, 8, 229; 10, 351; 16, 325.

⁵ Naftel, J. A., Ind. and Eng. Chem. Anal. Ed., 1939, 11, 407.

during the preliminary heating of the material. The overhead heater was also used instead of the water bath described in the original method, for it was found that the temperature could be much more easily controlled by this means. First, a survey was made of purified natural foodstuffs used in the diet of the rat. The foods containing the smallest amounts of boron were selected and the removal of their boron was attempted. Strong acid and methyl alcohol were used for this purpose in an attempt to remove the boron in the form of the methyl borate. Investigation of the extraction of boron with methyl alcohol showed first that quantitative recovery of boron used by this means was seldom obtained. By increasing the number of extractions or decreasing the amount of food to be extracted the results were not improved. Furthermore, this procedure was found unsatisfactory, first because complete elimination of the element appeared impossible, and secondly, in the cases where the boron was largely separated from the food, particularly in the case of protein, the food was so altered in its nutritive properties that it was not satisfactory for nutritional studies. It was evident at this stage, therefore, that the best solution to this problem was the preparation of an adequate diet composed of foodstuffs as low in boron as could possibly be found without attempting the removal of the element.

At about this time the report of Hove and associates⁶ on their studies of boron in animal nutrition appeared. Observations were then available in this laboratory, using a diet in connection with an experiment conducted for other purposes, but the boron content of which was practically the same as that of the Wisconsin experimenters. The various components of this diet along with others used in our laboratory had been tested for boron. The composition of this ration was considerably different from that used by Hove and his co-workers.⁶ Its total boron content was 163 µg per kg as compared with 155 µg of the diet described by the above mentioned investigators.⁶ It was thought, therefore, that it might be of interest to record our findings as further evidence of the results already reported,⁶ particularly since the animals fed the boron-low diet in this laboratory have been observed for a longer period of time and in somewhat greater detail.

The lactalbumin was prepared by the Harris Laboratories from fresh centrifuged milk. It is a product of high chemical purity, its ash content being but 0.72%. It was found to be extremely low in boron. Wheat gluten and gelatin contained about the same concentration

⁶ Hove, E., Elvehjem, C. A., and Hart, E. B., Am. J. Physiol., 1939, 127, 689.

Composition of Diet Lactalbumin 10.0 Wheat gluten 4.0 Gelatin 4.0 Salt mixture No. 22 5.7 Purified sweet butter fat 8.0 Dextrose to 100 Viosterol 15 drops per kilo Thiamin hydrochloride — 20 µg per rat per day Liver concentrate \equiv 3.5 g fresh liver per rat per day Vitamin E concentrate 3 mg per rat per day	Composition of Salt Mixture CaCO ₃ CaHPO ₄ .2H ₂ O KCl MgSO ₄ NaH ₂ PO ₄ .H ₂ O NaCl NaI Ns ₂ SiO ₃ .9H ₂ O FeSO ₄ (NH ₄) ₂ SO ₄ .6H ₂ O CuSO ₄ .5H ₂ O MnSO ₄ .4H ₂ O ZnCl ₂	re No. 22 1.08 .72 .85 .50 1.14 1.24 .00015 .035 .07 .025 .005 .01 5.7 g a 100 g diet
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of boron as this highly purified lactalbumin; therefore these 3 were selected as the sources of protein for this diet. Dextrose was the carbohydrate found to be lowest in boron. Sweet butter fat remelted and filtered served as the source of fat and vitamin A. Viosterol, a liver concentrate; especially prepared by the Lederle Laboratories and vitamin E concentrate prepared by the Mackenzie method, did not, in the amounts analyzed, show the presence of boron. The salt mixture contributed the greatest amount of boron of any of the ingredients of this diet.

Litter mates weighing 35-40 g were used in this experiment. These animals were housed in galvanized wire cages in the regular animal room of this laboratory. Monel metal or porcelain feeding cups and soft glass‡ drinking tubes were used.

Growth and reproduction on this diet were studied.

Extensive histological studies were also made on the tissues of these animals,

Results. The growth in body weight was observed and the food consumption and water intake were recorded; the average daily intake for the experimental period of 34 weeks being 9.6 g of diet per male and 7.8 g per female. The average daily boron intake was $1.56 \,\mu g$ per male and $1.27 \,\mu g$ per female. The general appearance of these animals was good and the rate of growth was normal. The growth of rats on this ration is shown in Table I. It is of interest to note that during the first 6 weeks these animals averaged a daily food intake of $5.2 \, g$ containing $0.85 \, \mu g$ boron. This compares well with the observations of the Wisconsin experimenters.

[†] I wish to thank the Lederle Laboratories, Inc., for supplying this material.

⁷ Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., V. S. Public Health Rep., 1939, 53, 1779.

[‡] Common soft glass is reported to be free from boron.8

⁸ Berger, K. C., and Truog, E., Ind. and Eng. Chem., Anal. Ed., 1939, 11, 540.

TABLE I.
Growth of Rais on Boron-low Diet.

	No. of rats	Avg wt in g per day	Avg daily food consumption in g	No. weeks avg	
Males	12	3.4	9.6	34	
Females	11	2.2	7.8	34	

The females on this synthetic diet were allowed to carry through 3 litters each, averaging 7 young per litter which were born living, and normal in appearance and weight and which they successfully raised. Further matings were not carried out.

Detailed pathological studies of the tissues of the rats at the end of 34 weeks' experimental period showed that they were normal in every respect.

Since the diet has been used primarily in another investigation, the effect of supplementing it with boron in various concentrations has not been observed and, hence, no comparison can be made between the animals fed the diet described and rats receiving boron in greater concentrations.

The data presented here confirm the findings of Hove, Elvehjem and Hart⁶ that if boron is actually essential for growth and development of the rat, it must be in extremely small amounts since an average of $1.27-1.56 \mu g$ per rat daily for a period of 34 weeks apparently satisfied its requirement for normal function. It is possible that a deficiency of this element would become apparent in the second or later generations when the boron store might become markedly depleted.

Summary. A synthetic diet is described which has a boron content of 163 µg per kg. This diet supports good growth and development in the rat. Food consumption compares well with that of animals on good purified diets used in this laboratory. The reproductive processes are normal. The young born to mothers on this boron-low diet appear normal and are successfully raised. Pathological studies showed the tissues of these rats to be normal. No evidence is obtained under the experimental conditions that boron is essential in the nutrition of the rat.

11401

Urinary Excretion of Ascorbic Acid by the Rat as Influenced by Ingestion of Certain Carbohydrates.*

CLAIRE A. FREDERICK, N. B. GUERRANT, R. ADAMS DUTCHER AND C. A. KNIGHT.

From the Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

It is now recognized that the rat is not only able to subsist indefinitely on a diet that is markedly scorbutogenic to the guinea pig, but while doing so, stores in its tissues and excretes in its urine measurable amounts of ascorbic acid. Workers in this field are not in complete agreement regarding the relationship of the composition of the ingested diet to the amounts of ascorbic acid stored and excreted by the rat. Some investigators1,2 have contended that the composition of the diet is an influencing factor, while other investigators3-6 have failed to demonstrate that ascorbic acid output is affected by changes in dietary ingredients. Other investigators7-10 have contended that the ordinary constituents of the diet, such as sugar, fats and proteins, have no effect on the urinary excretion of ascorbic acid by the rat, but that high rates of excretion can be induced by feeding oats, oat oil, the unsaponifiable portion of oat oil, halibut liver oil and certain cyclic compounds of the terpene and sesqui-terpene series. Early reports by this group of investigators postulated the existence of a precursor from which the ascorbic acid was formed by the rat. In the later reports, however, these authors

^{*} Authorized for publication on March 16, 1940, as paper No. 962 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

¹ Hopkins, F. G., Slater, B. R., and Milliken, G. A., Biochem. J., 1935, 29, 2803.

² Menaker, M. H., January, 1938, Master's Thesis, Pennsylvania State College.

³ Svirbely, J. L., Am. J. Physiol., 1936, 116, 446.

⁴ Zilva, S. S., Biochem. J., 1936, 30, 857.

⁵ Scheunert, A., and Schieblich, M., Z. Physiol. Chem., 1937, 247, 272.

⁶ Mentzer, C., and Urbain, G., Compt. Rend. Soc. Biol., 1938, 128, 270.

Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., Science, 1938, 88, 552.

⁸ Longenecker, H. E., Musulin, R. R., and King, C. G., Proc. Am. Soc. Biol. Chem., J. Biol. Chem., 1939, 128, p. Ix.

⁹ Musulin, R. R., Tully, R. H., 3rd, Longenecker, H. E., and King, C. G., J. Biol. Chem., 1939, 129, 437.

¹⁰ Longenecker, H. E., Musulin, R. R., Tully, R. H., 3rd, and King, C. G., J. Biol. Chem., 1939, 129, 445.

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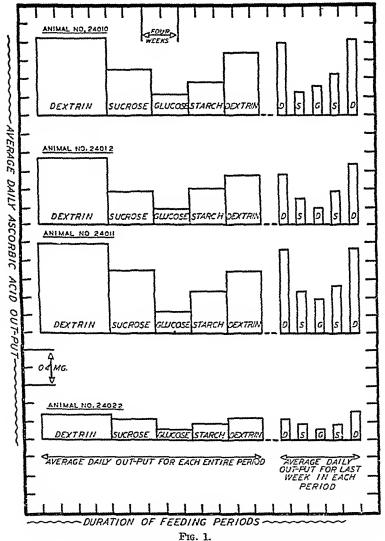
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Summary. A synthetic diet is described which has a boron content of $163 \mu g$ per kg. This diet supports good growth and development in the rat. Food consumption compares well with that of animals on good purified diets used in this laboratory. The reproductive processes are normal. The young born to mothers on this boron-low diet appear normal and are successfully raised. Pathological studies showed the tissues of these rats to be normal. No evidence is obtained under the experimental conditions that boron is essential in the nutrition of the rat.

In the instance of the first series of animals, all animals were fed the diet containing the dextrinized corn starch during the first period. This was followed by the sucrose-containing diet, the glucosecontaining diet and the starch-containing diet, respectively. At the termination of the studies with these diets, the animals were again fed the dextrinized corn starch diet for a period of several weeks.



Amounts of ascorbic acid eliminated in the urine of rats while receiving diets similar in composition, the only difference being in the type of carbohydrate which they contained.

abandon this view and postulate that the ascorbic acid is formed through intermediary metabolism, a view previously expressed by other investigators.⁶

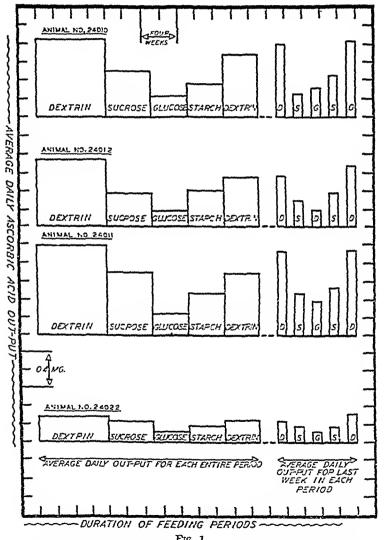
Because of the conflicting reports concerning the possible origin of ascorbic acid in the body of the rat, as well as those concerning factors influencing its elimination, it seemed desirable that our previous studies be repeated, especially those relating to the carbohydrate portion of the diet. The present report contains some of the data obtained in the course of the latter investigation.

Experimental. In our studies we have used half-grown rats as the experimental subjects. In order to collect the urine quantitatively, the animals were maintained in individual, cylindrical, galvanized wire cages, each of which was suspended above a 10-inch glass funnel. Beneath the funnel was placed a small glass vessel containing, as a preservative, 4 ml of metaphosphoric acid solution (10%) to which had been added a trace of 8-hydroxy-quinolin and a one-fourth-inch layer of paraffin oil. The cages were provided with galvanized wire bottoms with mesh of sufficient size to allow all fecal particles to pass through. Under each cage was placed a finer galvanized wire screen to prevent the fecal matter from entering the funnel. In order to further minimize contaminants, which might enter the funnel, the cages were provided with special food cups and drinking fountains. Each cage and its supplementary equipment were cleaned thoroughly at weekly intervals and, when necessary, the funnels were changed daily.

The collections of urinary samples were begun as soon as the animals were transferred to the metabolism cages and fed the experimental diet. The 24-hour collections of urine were removed at a definite time each day, their volumes recorded and aliquots titrated with Na 2,6-dichlorobenzenone indophenol solution (175 mg of the dye in 500 ml of hot water).

The diets used in these studies were composed of fat-free casein 18, salt mixture 3, Cell U flour 2, fat-free yeast 8 and fat-free carbohydrate 77 parts. In the majority of experiments, the fat-soluble vitamins were furnished as beta carotene and calciferol. In a few instances these vitamins were supplied by adding 2 parts of cod liver oil to the basal diet. The carbohydrates used were: dextrinized corn starch, raw corn starch, sucrose, glucose, and in a limited number of feeding periods of short duration, mannose, sorbose, fructose and lactose. The amount of food consumed daily by each animal was recorded and the amount fed was only slightly in excess of that consumed during the previous 24 hours.

In the instance of the first series of animals, all animals were fed the diet containing the dextrinized corn starch during the first period. This was followed by the sucrose-containing diet, the glucosecontaining diet and the starch-containing diet, respectively. At the termination of the studies with these diets, the animals were again fed the dextrinized corn starch diet for a period of several weeks.



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With subsequent series of animals, diets of similar composition were used, the experimental difference being in the sequence in which they were fed to the test animals.

Since space does not permit the presentation of all the data at this time, only the condensed data relating to the urinary ascorbic acid output of 4 typical animals from the first series are given

The duration of the various feeding periods were 8, 5, 4, 4 and 4 weeks, respectively. The urinary ascorbic acid elimination has been expressed as the average daily output for the entire period during which each of the respective diets was fed. In order to indicate the probable carry-over effect of one diet on the succeeding diet, the average daily urinary ascorbic acid output for the last week of each feeding period is given on the right of the graph (Fig. 1).

Discussion. With all of the animals used, the urinary ascorbic acid output was greatest while the animals were consuming the diet containing the dextrinized corn starch. This was found to be true irrespective of the sequence in which the diets were fed. On the other hand, in most instances, these same animals eliminated the smallest quantity of ascorbic acid while consuming the glucosecontaining diet. However, the frequency of the latter observation seemed to depend somewhat on the sequence in which the glucose diet was fed.

The greater ascorbic acid elimination resulting from the consumption of the diet containing the dextrinized corn starch as compared to the other diets, does not appear to be readily explainable from the data at hand. While the average daily consumption of the dextrinized corn starch was slightly greater than the consumption of the other diets and while there seemed to be a slight correlation between daily food intake and the amount of urinary ascorbic acid, this observation is sufficient only to explain a small portion of the increase in ascorbic acid elimination. The inadequacy of such an explanation can be readily observed from the fact that animal No. 24022 consumed as much of the respective diets as did animal No. 24011 and, while doing so, eliminated only about one-third as much urinary ascorbic acid as did the latter animal. However, it may be stated that the relative amounts of ascorbic acid eliminated by the various animals while receiving the sequence of diets were of the same order of magnitude for the respective diets.

The question as to why greater amounts of ascorbic acid were excreted, while the rats were consuming the dextrinized corn starch diet, than when the raw corn starch diet was consumed, cannot be

answered at this time. The possibility of the existence of different amounts of an ascorbic acid precursor in the two diets does not seem tenable, at least in this instance. To verify this point, some of the rats, after having been returned to the dextrinized corn starch diet for several weeks, were given weighed amounts (25, 50 or 100 mg) of carvone (Eastman No. 1094) to determine the effect of this substance on ascorbic acid output. Since it was found impossible to determine, quantitatively, the amount of this volatile substance actually consumed by the rats when it was mixed with the diet, the carvone was diluted with olive oil and given by stomach tube. However, with this procedure, the tests proved unsatisfactory in that the animals began to lose weight after the administration of the first or the second dose of carvone and the majority of the test animals died within 6 or 7 days. In no case was there more than a twofold increase in the amount of ascorbic acid eliminated in any one day and such increases did not remain consistent from day to day.

Conclusions. It is apparent that the amount of ascorbic acid eliminated in the urine of the rat depends upon at least 2 major factors, namely, the type of carbohydrate ingested by the rat, and the physiological variations within the rat itself. Since it was frequently found that ascorbic acid elimination varied as much with different animals as it did with different diets, it is evident that the latter factor must be given due consideration. The data submitted, however, do not explain the origin of the ascorbic acid nor do they explain the differences in the amounts of ascorbic acid excreted in the urine of different animals while receiving comparable amounts of the same diet. A number of theoretical possibilities suggest themselves but these offer no immediate solution to the problem. It would serve no useful purpose to say that the ascorbic acid is probably of endogenous origin or that it has its origin in intermediate metabolites. Such suggestions fall far short of explaining the origin of the ascorbic acid in the body of the animal and would be equally ineffective in explaining why different animals excreted different amounts of this substance while consuming comparable portions of the same diet.

11402

Loss of Carbohydrate Metabolism Factor During Boiling of Vegetables.*

LAURENCE G. WESSON. (Introduced by Veader Leonard.)

From the Veader Leonard Laboratory of Experimental Therapeuties,

Baltimore, Md.

Abnormally high respiratory quotients during carbohydrate assimilation are given by rats that have been maintained for a number of months on a fat-deficient diet. These high quotients, a large proportion of them above 1, have been attributed to a lack, in the fat-deficient diet, of an accessory factor that is necessary for normal carbohydrate metabolism. The

In the present paper it will be shown that rats maintained for 3 months or longer on a diet of boiled vegetables give carbohydrate RQ's that are abnormally high as compared with animals on a stock diet. This is interpreted as indicating that the boiled vegetable diet is likewise deficient in this carbohydrate metabolism factor. As it has previously been shown that raw vegetables and fruits apparently possess a high content of this factor, it now becomes evident that a loss of this factor takes place during the boiling of vegetables. That this is the case is confirmed by feeding rats that show the abnormal carbohydrate metabolism the same vegetable diet, but autoclaved instead of boiled. The effect of the autoclaved diet on these rats will be shown to be similar to that obtained by feeding the ether-soluble substances extracted from raw vegetables and animal fats in previous investigations. 1.2.7, namely, the restoration of the carbohydrate assimilatory RQ's to nearly normal.

Diets. Boiled vegetable diet. A minced mixture of 2 parts by weight of potatoes, 1 part of carrots, and 1 part of string beans, to which was added 0.25% NaCl and a surplus of tap water, was

^{*} Presented in abstract form before the American Society of Biological Chemists, at Baltimore, April 2, 1938 (J. Biol. Chem., 1938, 123, exxv).

¹ Wesson, L. G., J. Biol. Chem., 1927, 73, 507.

² Wesson, L. G., and Burr, G. O., J. Biol. Chem., 1931, 91, 525; Wesson, L. G., J. Biol. Chem., 1933, 100, 365; Wesson, L. G., and Murrell, F. C., J. Biol. Chem., 1933, 102, 303; Proc. Soc. Exp. Biol. And Med., 1934, 31, 1118.

[†] Previously unpublished data supporting this statement: Using a group of 4 rats in each case, the average maximum carbohydrate assimilatory RQ before dosing with approximately 0.3 g of the ether-soluble substances of raw potato was 1.08, after dosing it was 0.97; of raw carrot, 1.05 before dosing, and 0.89 after dosing; of raw apple, 1.05 before, and 1.01 after dosing.

actively boiled, with frequent stirring, for 8 hr in an open saucepan. At the end of that time, the mixture was of a pasty consistency, and of somewhat less than the original volume. Before the mixture cooled, 10 ml of an aqueous 5% solution of Na benzoate per 500 ml (0.1%) was stirred into it. It was then refrigerated until fed to the rats. In addition to the boiled vegetable mixture ad libitum, approximately 0.5 g of ether-extracted brewers' yeast per rat was fed daily during most of the work.

Autoclaved vegetable diet. The same vegetable mixture that was used in the preparation of the boiled vegetable diet, without the addition of water, was heated in sealed jars for 4 hr at 15 lb steam pressure.

Procedures. The respiratory exchange was determined by the use of a closed-circuit calorimeter of the type previously employed for this purpose.³ The dextrin test meal following 18 to 20 hr of fasting was the same as that described in previous papers on this subject.^{2,3}

Results. Six male and 5 female rats, 6 months old, were used. Approximately 3 months' feeding of the boiled vegetable diet was required before the first indication of abnormality in the carbohydrate metabolism was observed. This abnormality is shown in Table I by a significant difference between the carbohydrate assimilatory RQ's of these 11 rats and the corresponding RQ's of rats on a stock diet. The high RQ's are distinctly abnormal and are similar to those of rats on a fat-deficient, purified diet.²

In Table II are given the average carbohydrate RQ's of 6 of the abnormal rats before and after 7 days' feeding of the autoclaved diet. These values show that a marked lowering of the RQ's is

TABLE I.

RQ's Following Dextrin Test Meals with Rats on a Stock Ration and on a Boiled

Vegetable Diet.

Time after test meal hr		ration3 on 10 rai	ts	Boiled vegetable diet 82 runs on 11 rats				
	Mean RQ	RQ's No.	> 1.00 Max.	Mean RQ	RQ's	> 1.00 Max.		
1.5	.90 ± .06*	0	1.00	1.00 ± .005	31	1.09		
2.5	$.91 \pm .02$	0	1.60	$1.01 \pm .004$	50	1.09		
3.5	$.89 \pm .02$	0	0.99	$1.03 \pm .005$	59	1.11		
4.5	$.88 \pm .02$	0	1.00	$1.01 \pm .007$	58	1,11		
5,5	$.85 \pm .02$	0	1.00	$0.94 \pm .009$	17	1.04		

^{*}Standard deviation of the mean.

³ Wesson, L. G., J. Nutr., 1931, 3, 503.

11402

Loss of Carbohydrate Metabolism Factor During Boiling of Vegetables.*

LAURENCE G. WESSON. (Introduced by Veader Leonard.)

From the Veader Leonard Laboratory of Experimental Therapeutics,

Baltimore, Md.

Abnormally high respiratory quotients during carbohydrate assimilation are given by rats that have been maintained for a number of months on a fat-deficient diet.^{1,2} These high quotients, a large proportion of them above 1. have been attributed to a lack, in the fat-deficient diet, of an accessory factor that is necessary for normal carbohydrate metabolism.^{1,2}

In the present paper it will be shown that rats maintained for 3 months or longer on a diet of boiled vegetables give carbohydrate RQ's that are abnormally high as compared with animals on a stock diet. This is interpreted as indicating that the boiled vegetable diet is likewise deficient in this carbohydrate metabolism factor. As it has previously been shown it that raw vegetables and fruits apparently possess a high content of this factor, it now becomes evident that a loss of this factor takes place during the boiling of vegetables. That this is the case is confirmed by feeding rats that show the abnormal carbohydrate metabolism the same vegetable diet, but autoclaved instead of boiled. The effect of the autoclaved diet on these rats will be shown to be similar to that obtained by feeding the ether-soluble substances extracted from raw vegetables and animal fats in previous investigations. in anely, the restoration of the carbohydrate assimilatory RQ's to nearly normal.

Diets. Boiled vegetable diet. A minced mixture of 2 parts by weight of potatoes, 1 part of carrots, and 1 part of string beans, to which was added 0.25% NaCl and a surplus of tap water, was

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¹ Wesson, L. G., J. Biol. Chem., 1927, 73, 507.

² Wesson, L. G., and Burr, G. O., J. Biol. Chem., 1931, 91, 525; Wesson, L. G., J. Biol. Chem., 1933, 100, 365; Wesson, L. G., and Murrell, F. C., J. Biol. Chem., 1933, 102, 303; Proc. Soc. Exp. Biol. And Med., 1934, 31, 1118.

[†] Previously unpublished data supporting this statement: Using a group of 4 rats in each case, the average maximum carbohydrate assimilatory RQ before dosing with approximately 0.3 g of the ether-soluble substances of raw potato was 1.08, after dosing it was 0.97; of raw carrot, 1.05 before dosing, and 0.89 after dosing; of raw apple, 1.05 before, and 1.01 after dosing.

11403

Antistreptolysin Values in the General Population of Puerto Rico.

P. Morales-Otero and A. Pomales-Lebrón.

From the Department of Bacteriology of the School of Tropical Medicine, San Juan, Puerto Rico.

While studying the antistreptolysin content of the blood in cases of recurrent tropical lymphangitis¹ we were strongly impressed by the difference in values shown by these and by apparently normal cases. The question came up as to what should be considered as normal value for the general population under our local conditions.

Coburn and Pauli² determined the natural level of antistreptolysin in human blood, selecting for study a group of student nurses entering training at the Presbyterian Hospital, New York, on September 1932. These subjects were kept under clinical observation. Throat cultures were taken during periods of respiratory infection and antistreptolysin determinations were made at different intervals. The findings were classified in several groups: (a) those who contracted pharyngitis due to hemolytic streptococcus infection, (b) those who contracted other infections, the agent being unknown, (c) those who appeared to escape infection with hemolytic streptococci.

At the beginning of their study the median value for 30 individuals was 63 units. Only 5 subjects had a titer higher than 100 units. Ten subjects infected with hemolytic streptococci showed a subsequent rise in titer. None of those who escaped infection developed a significant rise in titer. The median titer of the group that escaped infection was 50 units 18 months later. These observations were interpreted by the authors to mean that the natural human antistreptolysin value is ordinarily about 50 units.

In order to determine the antistreptolysin values in our general population we proceeded to study a number of individuals, making the following classification: (1) apparently normal throats with no history of streptococcus infection and from which hemolytic streptococci could not be cultured at the time the determination was made, (2) apparently normal throats from which hemolytic streptococci were cultivated, and (3) apparently normal throats with a definite

¹ Morales-Otero, P., and Pomales-Lebron, A., P. R. J. Pub. Health and Trop. Med., 1936, 12, 43.

² Coburn, A. F., and Pauli, R. H., J. Exp. Med., 1935, 62, 129.

TABLE II.

Lowering of Abnormal Dextrin RQ's of 6 Rats on a Boiled Vegetable Diet, Followed by 7 Days' Feeding of the Autoelaved Diet.

Time after test meal hr	Boiled veg 6 runs e	getable di on 6 rats	Autoclaved vegetable diet 6 runs on 6 rats			
	Mean RQ		> 1.00 Max.	Mean RQ	RQ's	> 1.00 Max.
1.5 2.5	1.02 ± .02 1.02 ± .01	3 5	1.0S 1.07	.93 ± .02 .94 ± .02	1	1.01 0.98
3.5 4.5	1.07 ± .01 1.01 ± .02	6 5	1.11 1.04	$.95 \pm .01$ $.97 \pm .02$	0 1	0.98
5.5	$0.90 \pm .02$	0	0.99	$.94 \pm .01$	0	0.98

produced by the autoclaved diet, similar to the effect of animal fats and of the ether-soluble substances of raw vegetables and fruit.1,2,†

Conclusions. A loss of the carbohydrate metabolism factor takes place during the boiling of vegetables in an open vessel. This makes plausible the possibility that some deficiency of this factor occurs in many human dietaries. Since its lack causes abnormal fat formation and a disturbance of the carbohydrate metabolism of rats, it seems reasonable to consider a possible dietary deficiency of this factor in the study of prediabetic obesity.

Summary. Abnormally high respiratory quotients during carbohydrate assimilation are given by rats that have been maintained on a diet of boiled vegetables. This indicates a loss of an appreciable part of the carbohydrate metabolism factor which the vegetables contain in the unboiled condition.

The question is raised as to a possible causative connection of a dietary deficiency of this factor with prediabetic obesity.

population of Puerto Rico. The mean value of 136 apparently normal persons from which hemolytic streptococci could not be cultured at the time the determination was made was 84.1 units. In 43 apparently normal subjects harboring hemolytic streptococci at the time the determination was made, the mean antistreptolysin value was 144.9 units. In 96 normal persons, not harboring demonstrable hemolytic streptococci at the time the determination was made, but having a definite past history of streptococcus infection, the mean value was 154.5 units. The mean value for the 3 groups was 127.8 units.

11404 P

Effects of Renin and of Angiotonin Upon Isolated Perfused Heart.

W. H. PHILIP HILL* AND E. COWLES ANDRUS. (Introduced by W. T. Longcope).

From the Department of Medicine, Johns Hopkins University and Hospital.

The authors have examined the influence of renin. and of angiotonin, upon the isolated hearts of cats perfused with Ringer-Locke solution by the Langendorff method. Renin was prepared by alcohol precipitation of fresh pig's kidney cortex and fractional precipitation with ammonium sulphate, followed by prolonged dialysis. Angiotonin was prepared by the method of Page' and Helmer. The pH of this solution was adjusted to 7.0 with dilute sodium hydroxide. It was injected, in doses shown to produce minimal to large pressor effects in intact animals, into the stream of the perfusate just above the heart.

Remin. The observations of Tigerstedt and Bergman² and of Hessel³ that renin is without influence upon the isolated heart, were entirely confirmed by 33 injections in 16 experiments.

Angiotonin. Coronary Flow. Twenty-four injections in 12

^{*} Working under the Jacques Loeb and Archibold Fellowships.

i The authors are indebted to Dr. Page for his kindness in furnishing a quantity of angiotonin for comparison with that prepared by us.

¹ Page, L. H., and Helmer, O. M., J. Exp. Med., 1940, 71, 29.

² Tigerstedt, R., and Bergman, P. G., Skand. Arch. Physiol., 1898, 8, 223.

³ Hessel, G., Klin. Wehnschn., 1935, 17, 843.

history of previous streptococcus infection from which hemolytic streptococci could not be cultured.

Material and Methods. The material and methods used in this work were the same as those previously employed by us. The streptolysin was standardized, using standard Todd's globulin kindly supplied to us by Dr. Coburn. Single determinations were made in each case. Throat cultures and blood specimens were taken at the same time.

Results. One hundred and thirty-six determinations were made on Group 1. The lowest value was 18 units and the highest 150, with an average of 84.1 units.

Forty-three determinations were made on Group 2.* The lowest determination in this group was 48 units and the highest 350, with an average of 144.9 units.

Ninety-six determinations were made on Group 3. The lowest determination in this group was 37 units and the highest 333, with an average of 154.5 units.

Summary. Two hundred and seventy-five antistreptolysin determinations were made in apparently normal persons from the general

TABLE I.

Antistreptolysin Values in the General Population of Puerto Rico.

Antistreptolysir titer	No. of individuals	Group 1	Group 2	Group 3
0-20	1	1	0	0
21-40	Ð	8	0	i
41.60	40	31	4	5
61-80	34	28	2	4
81-100	43	32	4	7
101-120	30	9	8	13
121-140	38	18	6	14
141-160	32	9	7	16
161-180	12	0	2	10
181-200	12	0	2	10
201-250	13	Ó	4	9
251-300	6	0	2	4
301-400	4	Ō	2	2
401-500	1	Ō	0	1
			_	

Group 1—Consists of persons of apparently normal throats with no history of streptococcus infection and from which hemolytic streptococci could not be cultured.

Group 2—Consists of persons of apparently normal throats from which hemolytic streptococci were cultivated.

84.1 units

144.9 units

154.5 units

Group 3—Consists of persons of apparently normal throats with a definite history of previous streptococcus infection from which hemolytic streptococci could not be cultured.

Mean antistreptolysin titer by groups 8 Mean titer for the 3 groups: 127.8 units.

^{*} Strains belong to Groups A, C and G.

population of Puerto Rico. The mean value of 136 apparently normal persons from which hemolytic streptococci could not be cultured at the time the determination was made was 84.1 units. In 43 apparently normal subjects harboring hemolytic streptococci at the time the determination was made, the mean antistreptolysin value was 144.9 units. In 96 normal persons, not harboring demonstrable hemolytic streptococci at the time the determination was made, but having a definite past history of streptococcus infection, the mean value was 154.5 units. The mean value for the 3 groups was 127.8 units.

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Tigerstedt, R., and Bergman, P. G., Sland. Arch. Physiol., 1898, 8, 223.
 Hessel, G., Klin. Wohnschn., 1938, 17, 843.

experiments reduced the coronary output by from 27% to 86% (average 45%) in the presence of normal sinus rhythm. Four injections in one preparation were without result. The effect was maximal within 30 to 75 seconds after the beginning of the injection and lasted for one to 6 minutes. It showed no tendency to diminish with successive injections, though not more than 4 were administered to any one preparation. Four injections in 3 preparations, in which ventricular fibrillation was maintained by faradic stimulation of the ventricle, brought about a decrease in coronary flow by from 16% to 48% (average 28%). A late rise in coronary flow (averaging 16% for all injections) was frequently recorded. This late increase in flow was measurably reduced with successive injections.

Amplitude of beat. The amplitude of ventricular contraction was increased following every injection by from 18% to 300% (average 92%). This commenced later than the slowing of coronary flow, reached its maximum in 1 to 3 minutes, and persisted for 3 to 15 minutes. The effect of angiotonin upon amplitude of beat showed no consistent tendency to diminish with successive injections.

Heart Rate. Significant effects upon the heart rate were not recorded. In some experiments the rate of beat diminished slightly during the period of reduced coronary flow.

Summary. Upon the isolated hearts of cats perfused with Ringer-Locke solution renin produced no significant effect. Angiotonin on the other hand brought about decrease in coronary flow and increase in amplitude of beat, but no consistent effect upon heart rate.

11405 P

Pituitary Function in Parabiotic Triplet Rats.

R. O. GREEP.

From the Division of Pharmacology, Squibb Institute for Medical Research, New Brunswick, N. J.

Rat ovaries that have been made to function under the stimulus of the male pituitary as in the experiments of Goodman¹ (ovarian grafts), Witschi and Levine² (parabiosis) and Pfeiffer³ (mascu-

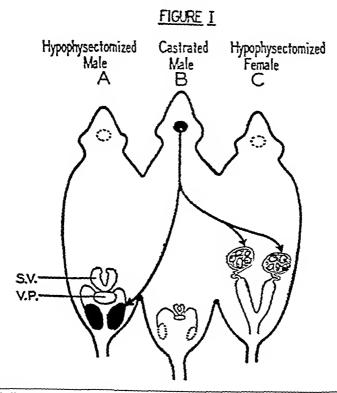
¹ Goodman, L., Anat. Rec., 1934, 59, 223.

² Witschi, E., and Levine, W. T., PROC. Soc. EXP. BIOL. AND MED., 1934, 32, 101.

³ Pfeiffer, C. A., Am. J. Anat., 1936, 58, 195.

linized female pituitaries) have uniformly failed to become luteinized. The luteinizing hormone, however, has been shown to be present in small amounts in the pituitaries of normal male rats and abundant in those of castrated animals (Hellbaum and Greep⁴). Cutuly, et al., have shown that the pituitary of a castrated male stimulates the interstitial cells as well as the seminiferous tubules of a parabiotic hypophysectomized male partner. Furthermore, there is considerable experimental evidence for assuming that the luteinizing hormone is identical with the substance responsible for the internal secretory function of the male gonad. The available evidence then is contradictory in that the male pituitary appears to secrete luteinizing hormone if the end organ is a male gonad, and only follicle-stimulating hormone if the end organ is a female gonad.

The present experiments were devised to bring the entire repro-



⁴ Hellbaum, A. A., and Greep, R. O., Am. J. Anat., 1940, in press.

⁵ Cutuly, E., McCullagh, D. R., and Cutuly, E. C., Endocrinology, 1937, 21, 241.

ductive systems of each sex under the simultaneous influence of a single male pituitary.

Male rats, aged 21 days, were gonadectomized and joined in parabiosis with 2 littermates—a male on one side and a female on the other. (See also Fig. 1 drawn like that of DuShane and others.") Four to 6 days later the 2 outside triplets were hypophysectomized. Antopsies were performed 7 to 16 days after hypophysectomy. The gonads, sexual accessories, thyroids and adrenal glands were weighed and examined grossly. Vaginal smears were followed in some instances. The observations reported are based on 8 sets of triplet parabionts in which all except the central animal had been completely hypophysectomized.

On the 7th day following the joining of these animals the vaginas of the hypophysectomized females opened and rapid testicular growth in the hypophysectomized male became apparent. The autopsy findings, illustrated by the data from a typical experiment (Table I), show that the principal physiological action of the castrated male pituitary on the ovary of a hypophysectomized female triplet parabiout is to promote the growth of follicles and cause the continuous secretion of estrogen as attested by persistent vaginal cornification and a distended uterus. The ovaries have not yet been examined microscopically for the presence of interstitial-cell or luteal stimulation but there was no evidence of luteinization by gross inspection. The hypophysectomized male parabiont, which had derived its gonadotropic stimulus from the same pituitary gland that produced only follicle growth in the female, showed a marked stimulation of the testes, and the secondary sexual structures, such as the prostate and sentinal vesicles, were greatly enlarged.

The secondary sexual structures of the central castrate remained entirely atrophic in all cases.

The peculiar ability of the castrated male rat pituitary to evoke

Organ Weights of 3 Animals Which Had Been Joined Together for 19 days. Rats "A" and "C" Had Been Hypophysectomized for 13 Days and the Central Animal "B" Had Been Castrated Since the Start of the Experiment.

Rat	Body wt at death, gm			Ventral prostate, mg	Ovaries, mg	Empty uterus, mg	Adrenals,	Thyroid, mg
Ā	52	1036	54	106			6.6 20.5	4.45 7.60
B	67 52	{	8	•	44.4	286	9.6	6.34
		C B	Locine W	T. Pfei	ffer. C. A.,	and Wi	tschi, E., P	ROC. SOC.

o DuSha , G. P., Levine, W. T., Pfeitfer, C. A., and Witschi, E., Proc. Soc

EXP. Bion. (MD MED., 1935, 88, 339.

a follicular response in a female and at the same time stimulate the testes of a male to secretory function is difficult to interpret considering the results which have been obtained with purified follicle-stimulating extracts of the pituitary. 7, 8, 9 Several explanations suggest themselves but all are purely conjectural: (a) the threshold for luteinization of the ovary may be far above that necessary to produce stimulation of testicular interstitial cells; (b) possibly the interstitial cell stimulating and luteinizing hormones are not identical; (c) it may be extremely difficult to cause luteinization of follicles which grow rapidly and become cystic.

11406 P

Efficacy of Pellets of Posterior Hypophysis and of Pitressin in Oil in Diabetes Insipidus.

JAMES A. GREENE AND L. E. JANUARY.

From the Department of Internal Medicine. State University of Iowa, College of Medicine, Iowa City.

The efficacy of the subcutaneous administration of dried posterior pituitary gland in diabetes insipidus has not been reported previously. Pellets of this material have been prepared and implanted subcutaneously into 4 cats with experimentally produced diabetes insipidus* and into 2 patients with diabetes insipidus. The results are shown in Table I. It is to be noted that mixing the material with tyrosine or impregnating the pellet with beeswax did not prolong the effect in the cats. An inflammatory reaction which occurred at the site of implantation of sterile pellets in the patients later required drainage. An attempt was made to prolong the action in man by impregnation of the pellets with lanolin or beeswax. reaction which developed at the site of implantation was so severe that the pellets had to be removed before complete absorption occurred.

Greep, R. O., and Fevold, H. L., Endocrinology, 1937, 21, 611.

⁸ Greep, R. O., Proc. Soc. Exp. Biol. and Med., 1939, 42, 454.

⁹ Chow, B. F., Greep, R. O., and van Dyke, H. B., J. Endocrinology, 1940, 1, 439.

^{*} Available through the courtesy of W. R. Ingram, Department of Anatomy, State University of Iowa, College of Medicine.

TABLE I.

Pluration of Effect of Dried Posterior Pituitary Gland Implanted Subcutaneously in Cats and in Man with Diabetes Insipidus.

			Contro	ol period			Poster	or pitui	tary pello	ets
		No. Days	Avg fluid intake, cc	Avg urinary output, cc	Avg urinary specific gravity		Days netive	Avg fluid intake, cc	Avg urinary output, cc	Avg urinary specific gravity
Cut	1	14		261	1.016	102.0	11		151	1.030
* *	2	6		580	1.006	98.0	6		231	1.019
7 7	3	7		488	1.008	190,0*	4		316	1.015
7.7	4	7		370	1.012	100,01	7		175	1.025
Patient	1	3	13,170	13,815	1.000	300.0	3	2,650	2,025	1.015
,,	2	4	11,300	11,925	1.000	300.0	3	1,400	1,283	1.015

*Pellet consisted of equal parts of tyrosine and dried posterior pituitary gland. †Pellet impregnated with beeswax.

The above results demonstrate that pellets of dried posterior pituitary gland implanted subcutaneously control the manifestations of diabetes insipidus, but that this method is not applicable for treatment in man. For this reason pitressin tanuate† in oil was employed. This material was administered to 3 cats with experimentally produced diabetes insipidus and to 3 patients with the syndrome. In the cats 1.0 cc ameliorated the manifestations for 3 to 7 days and in man for 30 to 82 hours. There were no unpleasant or deleterious general or local reactions. The symptoms of the disease have been controlled in the 3 patients by the subcutaneous injection of 1.0 cc every 36 to 57 hours.

t Supplied through the courtesy of Parke, Davis and Company.

11407 P

Rôle of the Sympathetic Nervous System in Experimental Neurogenic Hypertension.

KEITH S. GRIMSON. (Introduced by Dallas B. Phemister.)

From the Department of Surgery, University of Chicago.

Recent clinical studies1 have renewed interest in central or psychosomatic factors in essential hypertension. The sustained neurogenic hypertension in dogs described by Heymans and Bouckaert2 seems from this point of view to afford a better experimental approach to the problem of hypertension and sympathectomy than the renal hypertension described by Goldblatt,3 and shown to be uninfluenced by total sympathectomy.4-7 The recent demonstration. Grimson, Bouckaert and Heymans,8 that a sustained neurogenic hypertension of renal origin may be produced by a central reflex mechanism tends to correlate these two methods for producing experimental hypertension. The present study is based upon an effort to determine the blood pressure levels produced by section of the depressor nerves in normal dogs and compare them with the pressure levels produced by the same procedure in dogs sympathectomized with the exception of the nerve supply to the kidneys and adrenals, as well as to study the effects of renal denervation, splanchnic resection, and total paravertebral sympathectomy on the former group.

Heymans and Bouckaert⁹ have shown that section of the depressor nerves produces a persistent hypertension and that total sympathectomy eliminates the hypertension. Goldblatt, Kahn, Bayless and Simon¹⁰ have recently failed to obtain this type of hypertension

¹ Katz, L. N., and Leiter, Louis, Psychosomatic Med., 1931, 1, 101.

² Heymans, C., and Bouckaert, J. J., C. R. Soc. Biol., 1931, 106, 471; Bull. Acad. Roy. Med. de Belg., 1939, p. 441.

³ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W. J. Exp. Med., 1934, **59**, 347.

⁴ Alpert, L. F., Alving, A. S., and Grimson, K. S., Proc. Soc. Exp. Biol. and Med., 1937, 37, 1.

⁵ Freeman, N. F., and Page, I. H., Am. Heart J., 1937, 14, 405.

⁶ Heymans, C., Bouckaert, J. J., Bayless, F., and Samann, A., C. R. Soc. Biol., 1937, 126, 434.

⁷ Verney, E. B., and Vogt, M., Quart. J. Exp. Physiol., 1938, 28, 253.

⁸ Grimson. K. S., Bouckaert, J. J., and Heymans, C., Proc. Soc. Exp. Biol. and Med., 1939, 42, 225.

⁹ Heymans, C., and Bouckaert, J. J., C. R. Soc. Biol., 1935, 120, 82.

¹⁰ Goldblatt, H., Kahn, J. R., Bayless, F., and Simon, M. A., J. Exp. Med., 1940, 71, 175.

TABLE 1.

Duration of Effect of Dried Posterior Pituitary Gland Implanted Subcutaneously in Cats and in Man with Diabetes Insipidus.

			Contro	ol period		Posterior pituitary pellets				
		No. Days	Avg fluid intake, cc	Avg urinary output, cc	Avg urinary specific gravity	Wt of pellets,	Days active		Avg urinary output, cc	Avg urinary specific gravity
Cnt	1	14		261	1.016	102.0	11		151	1.030
3 1	23	6		580	1.006	98.0	6		231	1.019
**	3	7		485	1.008	190.0*	4		316	1.015
"	4	7		370	1.012	100.01	7		175	1.025
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From the Department of Surgery, University of Chicago.

Recent clinical studies1 have renewed interest in central or psychosomatic factors in essential hypertension. The sustained neurogenic hypertension in dogs described by Heymans and Bouckaert² seems from this point of view to afford a better experimental approach to the problem of hypertension and sympathectomy than the renal hypertension described by Goldblatt,3 and shown to be uninfluenced by total sympathectomy.4-7 The recent demonstration. Grimson, Bouckaert and Heymans,8 that a sustained neurogenic hypertension of renal origin may be produced by a central reflex mechanism tends to correlate these two methods for producing experimental hypertension. The present study is based upon an effort to determine the blood pressure levels produced by section of the depressor nerves in normal dogs and compare them with the pressure levels produced by the same procedure in dogs sympathectomized with the exception of the nerve supply to the kidneys and adrenals, as well as to study the effects of renal denervation, splanchnic resection, and total paravertebral sympathectomy on the former group.

Heymans and Bouckaert' have shown that section of the depressor nerves produces a persistent hypertension and that total sympathectomy eliminates the hypertension. Goldblatt, Kahn, Bayless and Simon¹⁰ have recently failed to obtain this type of hypertension

¹ Katz, L. N., and Leiter, Louis, Psychosomatic Med., 1931, 1, 101.

² Heymans, C., and Bouckacrt, J. J., C. R. Soc. Biol., 1931, 106, 471; Bull. Acad. Roy. Med. de Belg., 1939, p. 441.

³ Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W. J. Exp. Med., 1934, 59, 347.

⁴ Alpert, L. F., Alving, A. S., and Grimson, K. S., Proc. Soc. Exp. Biol. and Med., 1937, 37, 1.

⁵ Freeman, N. F., and Page, I. H., Am. Heart J., 1937, 14, 405.

⁶ Heymans, C., Bouckaert, J. J., Bayless, F., and Samaan, A., C. R. Soc. Biol., 1937, 126, 434.

⁷ Verney, E. B., and Vogt, M., Quart. J. Exp. Physiol., 1938, 28, 253.

⁸ Grimson. K. S., Bouckaert, J. J., and Heymans, C., Proc. Soc. Exp. Biol. and Med., 1939, 42, 225.

⁹ Heymans, C., and Bouckaert, J. J., C. R. Soc. Biol., 1935, 120, 82.

¹⁰ Goldblatt, H., Kahn, J. R., Bayless, F., and Simon, M. A., J. Exp. Med., 1940, 71, 175.

and Nowak and Walker¹¹ have stated that some hypertension follows depressor nerve section in sympathectomized dogs. These contraindications have further stimulated this study.

In 9 dogs both carotid simses were excised, the left vago-sympathetic-depressor nerve was cut, and a segment of the right sympathetic depressor trunk was removed. The control blood pressures were respectively 131, 133, 134, 134, 138, 138, 144, 148 and 174. These dogs were observed from 16 to 163 days after modulator nerve section and their pressure readings averaged respectively 238, 246, 239, 212, 194, 256, 257, 214 and 226. The late readings in 3 animals were appreciably higher than the early readings. Three animals had occasional readings of 280 to 300. None of the dogs failed to develop a hypertension and in none of them was there any late lowering of the hypertension.

In 6 normal dogs both paravertebral sympathetic chains were removed. Their control blood pressures averaged 142 and their pressures 14 to 29 days after sympathectomy averaged 112. Because of the observation (Grimson, Wilson and Phemister¹²) that sympathectomized dogs recover in a few months a new central vasomotor mechanism and restore their preoperative blood pressure the modulator nerves in these dogs were sectioned in the manner described above 14 to 29 days after the sympathectomy. Two dogs died shortly after operation without pressure elevation. The other 4 during the next 30 days had an average pressure of 98. No elevation was observed. Two were observed 90 and 108 days and developed pressures higher than before the sympathectomy, 164-206. Two other dogs sympathectomized 26 and 28 months previously and with restoration of their blood pressure to averages of 148 and 152 developed average pressures after modulator nerve section of 169 and 176 mm respectively with occasional readings of 200. This observation of levels higher than before sympathectomy suggests that the recovered central vasoconstrictor mechanism¹² is influenced by the modulator nerves.

Seven dogs* have now been sympathectomized with the exception of the splanchnic supply to the adrenals and kidneys according to the described technic. Their blood pressures just preceding modulator nerve section averaged 136 and during several weeks afterward 195. This elevation is definitely less than the average of the nine normal dogs with modulator nerve section described above which

¹¹ Nowak, S. J. G., and Walker, I. J., New England J. Mcd., 1939, 220, 269.

¹² Grimson, K. S., Wilson, H., and Phemister, D. B., Ann. Surg., 1937, 106, 801.

^{*} Three observed in Prof. C. Heyman's laboratory in Ghent,

was 231. Renal denervation in four of these 7 dogs has restored their pressure to about the normal level.

Further experiments have shown that renal denervation alone neither prevents nor appreciably alters the hypertension produced in normal dogs by modulator nerve section. They have also confirmed the observations of Nowak and Walker¹¹ that abdominal sympathectomy and division of the splanchnic nerves as well as complete sympathectomy except for one thoracic chain fails to restore the blood pressure of neurogenic hypertensive dogs to normal. Total sympathectomy as described above lowered the pressure of 3 neurogenic hypertension dogs from 239, 226 and 246 to 101, 122 and 91 respectively during 30, 18, and 40 days of observation. After 30 and 40 days in 2 of these animals blood pressure recovery was evident and progressed toward a moderate hypertension level. This parallels but exceeds the recovery following paravertebral sympathectomy in normal dogs previously reported.¹²

11408 P

Renal Phosphatase in Experimental Nephropathies.*

OPAL E. HEPLER, J. P. SIMONDS AND HELEN GURLEY.
From the Department of Pathology, Northwestern University Medical School.

The specific function of the rich phosphatase content of the kidney is still unknown. Since the kidney is almost invariably involved in metastatic calcification and is often the site of pathologic calcification it seemed possible that by comparing the location of the deposits of lime salts in these conditions with that of the phosphatase something might be learned concerning the relation of this enzyme to renal function.

For this purpose we studied phosphatase, acting optimally at a pH of about 9.0 on sodium glycerophosphate, in the kidneys of normal dogs and of dogs in which a toxic nephrosis has been produced by uranium nitrate, potassium bichromate and bichloride of mercury. We compared sections stained for phosphatase by Gomori's' method with the quantity of the enzyme obtained in aqueous extracts of the cortical tissue of the same kidneys as determined by Bodansky's

^{*} Aided by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

¹ Gomori, G., PROC. Soc. Exp. BIOL. AND MED., 1939, 42, 23.

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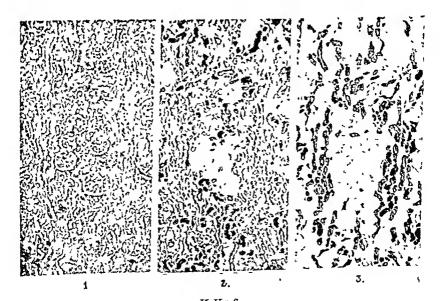
For this purpose we studied phosphatase, acting optimally at a pH of about 9.0 on sodium glycerophosphate, in the kidneys of normal dogs and of dogs in which a toxic nephrosis has been produced by uranium nitrate, potassium bichromate and bichloride of mercury. We compared sections stained for phosphatase by Gomori's' method with the quantity of the enzyme obtained in aqueous extracts of the cortical tissue of the same kidneys as determined by Bodansky's

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¹ Gomori, G., PROC. Soc. EXP. BIOL. AND MED., 1939, 42, 23.

chemical method. We soon found, however, that in kidneys in which pathologic calcification was present, there was no correlation between the quantity of phosphatase revealed by the chemical method and the microscopic picture in the Gomori-stained sections.

We therefore stained 3 consecutive sections of kidneys of the dogs used in these experiments, one with routine hematoxylin and eosin, one with Gomori's stain for phosphatase and one for calcium phosphate only by von Kossa's method. The routine sections revealed the location of necrotic or otherwise damaged tubular epithelium. Sections stained by Gomori's method showed all of the calcium phosphate present in approximately quantitative relations. Normally, phosphatase is present in the marginal zone, next the lumen, of the epithelium lining the proximal convoluted tubules. It is most abundant in the first two-thirds or three-fourths of these tubules, that is, in the labyrinth; less abundant in the straight terminal portion, that is, in the medullary rays and in the outer stripe of the outer zone of In Gomori-stained sections the calcium was more abundant and stained more deeply where it had been precipitated in normal or only slightly damaged cells by the action of the phosphatase during the process of staining than in those regions where its presence was the result of nathologic changes. Sections stained



K Hg 6. 1. H. & E. 2. Gomori. 3. Kossa. One injection—3 mg. HgCl₂ per 100 cc blood. Died 78 hours later.

by von Kossa's method revealed only that preformed calcium phosphate which was the result of pathologic calcification. In our experiments, this was present chiefly in the medullary rays in amounts varying with the length of survival of the animal, while the labyrinth was either free or showed only minute amounts (Fig. 1).

Calcification was found to occur early (28 hours after injection) and abundantly in the kidneys of dogs given a single intravenous injection of bichloride of mercury equal to 3 mg per 100 cc of blood. This dose of bichloride causes very severe injury to the epithelium of the straight distal portion of the proximal convoluted tubules, but no visible injury to the glomeruli. Doses of potassium bichromate and uranium nitrate that induced marked necrosis of the tubular epithelium have not been found to induce pathologic calcification in the kidneys of animals that have survived for 3 days.

None of the poisons (uranium, bichromate, bichloride of mercury) in the doses used appear to inactivate the phosphatase although they may kill the cell that contains it. This conclusion is confirmed by the results of quantitative determinations of the phosphatase in these kidneys by Bodansky's method and by study of stained sections. Necrotic and desquamated tubular epithelium of kidneys of dogs poisoned by bichloride stains diffusely by Gomori's method instead of deeply along the lumenal margin. Granular material stained by Gomori's but not by von Kossa's method in the capsular space of some glomeruli is continuous with similar material in the tubule which takes origin from such a glomerulus. It is believed to be cellular debris containing active phosphatase which has been regurgitated into the capsular space from the damaged tubule. debris accumulated in the straight terminal portion of the proximal convoluted tubules above the narrow part of Henle's loop stained both by Gomori's and von Kossa's method, and appears, therefore, to contain both active phosphatase and preformed calcium phosphate.

11409

Metabolism of Free Citrie Acid in the Rat.*

CARL A. KUETHER, CURTIS E. MEYER AND ARTHUR H. SMITH.
From the Department of Physiological Chemistry, Wayne University College of
Medicine, Detroit.

Studies in which pure citric acid was given by mouth to human subjects have shown that citric acid may be almost completely metabolized. In the dog, large amounts of orally administered citric acid are likewise destroyed, less than 1% of the compound given appearing in the urine and no "extra" citric acid in the feces. Similar results have been obtained with rabbits and swine. The possibility that the citric acid administered was not absorbed was precluded by Langecker who found a prompt and prolonged rise in the level of blood citrate and showed further that the enzymes and bacteria present in the intestinal tract of the rabbit do not destroy citric acid even when incubated for as long as 9 hours. The following experiments were conducted in order to determine whether or not the rat also possesses the ability to metabolize free citric acid.

A series of rats maintained in metabolism cages was fed the following diet ad libitum: lactalbumin (Borden No. 15-42) 15.0, dextrin 50.5, hydrogenated fat (Crisco) 27.0, cod liver oil (Mead) 5.0, and salt mixture¹⁰ 2.5%. This diet was supplemented daily with 2 cc of a solution containing 80 mg of liver extract (Lilly 343) and 200 mg of Ryzamin-B (Burroughs Wellcome).

The cages were placed over large glass funnels containing a 3/16

- 1 Östberg, O., Skand, Arch. f. Physiol., 1931, 62, 81.
- 2 Kuyper, A. C., and Mattill, H. A., J. Biol. Chem., 1933, 103, 51.
- 3 Boothby, W. M., and Adams, M., Am. J. Physiol., 1934, 107, 471.
- 4 Gonce, J. E., and Templeton, H. L., Am. J. Dis. Child., 1930, 39, 265.
- 5 Schuck, C., J. Nutrition, 1934, 7, 691.
- 6 Sherman, C. C., Mendel, L. B., and Smith, A. H., J. Biol. Chem., 1936, 113, 247, 265.
 - 7 Laugecker, H., Biochem. Z., 1934, 273, 43.
 - 8 Fürth, O. von, Minnibeck, H., and Edel, A., Ibid., 1934, 269, 379.
 - 9 Woods, E., Am. J. Physiol., 1927, 79, 321.
 - 10 Hubbel, R. B., Mendel, L. B., and Wakeman, A. J., J. Nutrition, 1937, 14, 273.

^{*} The data herein presented are taken from a dissertation submitted by Carl A. Kuether in partial fulfillment of the requirements for the degree of Master of Science in Wayne University, 1940.

This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

inch mesh screen which separated the feces from the urine, which was collected in a flask containing about 20 cc of 5% sulfuric acid. The funnels were rinsed down daily with distilled water. Citric acid was determined by the method of Pucher. Sherman and Vickery. It the final measurement being made in a photelometer using a filter with maximum transmission at 4250 Å.

After an adjustment period of 4 days, urine samples were collected for four 2-day intervals followed by 3 similar periods during which the rats received by stomach tube 400 mg per day of citric acid dissolved in distilled water to make 2 cc. Three more control samples were then collected. The urine samples were diluted to 200 cc and 2 cc aliquots taken for analysis. Later another group of animals was treated by the same procedure except that citric acid determinations were run on both the urine and the feces.

In order to show that the citric acid administered was actually absorbed and not destroyed by the bacteria in the gut. the intestinal contents of two rats were removed. One lot was mixed with citric acid and divided into 4 samples, 2 of which were analyzed immediately for citric acid and the other 2 incubated at 37° for 44 hours in glass stoppered flasks to prevent evaporation. The pH of the other sample of intestinal contents was measured, citric acid added and the pH brought back to the same point by addition of NaOH. This second sample was then treated the same as the first, divided into 4 parts, 2 analyzed immediately and 2 incubated at 37° for 44 hours. The citric acid found in the unbuffered control and incubated samples was 64.7 and 62.7 mg per g respectively and in the buffered samples 62.4 and 62.5 mg per g, showing that the intestinal contents of the rat do not destroy citric acid either at the normal pH of 6.5 or at the pH of 3.3 obtaining after the addition of citric acid.

Urinary pH was measured with a glass electrode on several animals before and after citric acid feeding, the urines being collected under toluene in large test tubes. Only samples uncontaminated with food or feces were used. The values differed by no more than 0.1 pH.

In Table I, the first line for each rat is the average intake and excretion of citric acid for three 2-day control periods immediately preceding the experimental periods. During these control periods each rat was excreting more citric acid in the urine than it was absorbing from the gut and consequently must have been synthesizing citric acid (Smith and Meyer¹²). The following 3 lines for

¹¹ Pucher, G. W., Sherman, C. C., and Vickery, H. B., J. Biol. Chem., 1936, 113, 235.

¹² Smith, A. H., and Meyer, C. E., J. Biol. Chem., 1939, 131, 45.

TABLE I. Disposition of Citric Acid by the Rat.

			ie acid i g per 2 (Citric acid excretion Mg per 2 days			<i></i>	ci	Exerction of citric acid of total intake		
Rat	Wt	Food	Extra	Total	Urine	Feces	Total		Urine	Feces	Total	
8	256	17.7	0	17.7	25.1	0.54	25,9	95.5	149	4.97	153	
		16.3	800	816	20.6	1.03	21.6	99.9	2.46	,123	2.58	
		12.9	600	613	31.8	0.56	32.4	99.8		,091	5,28	
		12.2	800	812	39.0	0.62	39,6	99.8		,076	4.88	
9	232	17.0	Ð	17.0	22.3	1.28	23.6	92.4	142	8.13	150	
		15.0	800	815	30,3	1.13	31.4	99.9	3.72	.139	3.86	
		12.2	800	812	32.8	0.95	33.8	99.9		.117	4.17	
		12.2	800	812	38.0	0.52	38.5	99.9	4.68	.064	4.74	
10	196	17.0	0	17.0	28.7	1.31	30.0	92.4	183	8.34	191	
		13.6	CON	614	19.1	1.20	20.3	99.8	3.11	.196	3.30	
		5,5	800	805	12.2	0.63	12.8	99.8	1.52	.078	1,60	
		5.5	800	805	13.6	0.56	14.2	0.9.9	1.69	.070	1.76	
11	156	10,2	0	10.2	19.3	0.43	19.7	96.1	197	4.39	201	
		4.8	Soo	805	15.7	3.49	19.2	99.6	1.96	.435	2.39	
		5,5	800	805	18.2	12,35	30.6	98.5	2.29	1.56	3.85	
		6.8	400	407	24.2	0.23	24.4	99.9	5.94	.056	6.00	
12	290	20.4	0	20,4	74.7	1.72	76.4	91.6	300	9.20	408	
		17.7	400	418	60.1	1.10	61.2	99.7	14.4	.264	14.7	
1		12.9	800	813	52.3	0.97	53.3	99.9	6.44	.119	6.56	
		12.9	800	S13	72.1	1.72	73.5	99.8	s.s ₆	.212	9.08	

each rat give the figures for individual periods during which the rats were receiving extra citric acid. During these periods, with one exception (Rat 11, 3rd period, when he had diarrhea) the absorption of the administered citric acid exceeded 99%, and the percentage of the absorbed citric acid which was excreted in the urine dropped to a very low value. In all 4 of the animals in which only urinary citric acid was followed, there was actually a decrease in the absolute amount of citric acid excreted following the feeding of free acid, and rats 10 and 12 in the table show the same result. In all of the animals, feeding of extra citric acid brought about a remarkable decrease in the percentage of the absorbed citric acid appearing in the urine, showing that under the experimental conditions imposed, the rat possesses the ability to metabolize free citric acid almost completely.

Summary. Citric acid administered to the rat is absorbed since no extra citric acid appears in the feces and intestinal contents do not destroy it. The albino rat has the ability to completely metabolize

maximum non-fatal quantities of free citric acid.

11410 P

Occurrence of Special Cell Groups at Vascular Pole of Glomerulus in Mammalian Kidneys.

WILLIAM KAUFMANN. (Introduced by Arthur W. Wright.)

From the Department of Pathology and Bacteriology, Albany Medical College, and the Laboratorics of the Albany Hospital, Albany, N. Y.

During the past year we have studied the juxtaglomerular corpuscles of Goormaghtigh¹⁻⁴ in normal and diseased human kidneys removed surgically and at autopsy. This material amounts at the present time to about 200 unselected cases. While similar structures were noted previously by others in laboratory animals,⁵ other mammals⁶ and selected human cases,^{7, 2} this is the first attempt to demonstrate agglomerations of peculiar cells or cell groups around the afferent arteriole of the glomerulus in routine autopsy and surgical material, stained by special as well as routine methods.

These corpuscles are composed of agglomerations of cells, which are situated chiefly at the vascular pole of the glomerulus between the macula densa⁶ of the distal convoluted tubule and the afferent arteriole. A thin layer of these cells may also surround the entire vessel Occasionally the cells extend along the first part of the arteriole as it enters the glomerular tuft. They usually occur outside the media, surrounding it like a sheath, but they may appear to compose the entire arteriolar wall. We have not observed the corpuscles in kidneys of stillborn infants or children up to 2 years of age. In certain diseased kidneys, as of arteriolar nephrosclerosis, benign or malignant, they may be hypertrophied and thus appear more conspicuous.

The cells which make up the corpuscles can be identified by their morphological characteristics as well as by their staining reactions. They are rather large, polygonal cells with indistinct cell outlines. They are frequently closely packed and delicate argyrophilic fibrils can often be detected between them. Masson's trichrome stain brings out peculiar, fine, fuchsinophilic granules in the cytoplasm, which is

¹ Goormaghtigh, N., Aich. Biol., 1932, 43, 575.

² Goormaghtigh, N., J. Physiol. 1937, 90, 1263.

³ Goormaghtigh, N., C. rend. soc. biol., 1936, 124, 293.

⁴ Goormaghtigh, N., and Handovsky, H., Arch. Path., 1938, 26, 1144.

⁵ Ruyter, J. H. C., Z. f. Zellforschung, 1925, 2, 242.

⁶ Zimmermann, K. W., Z. f. mikr. anat. Forsch., 1933, 32, 176.

⁷ Oberling, Ch., Ctcs. rend. hebd. Ac. Sciences, Paris, 1927, 184, 1200.

otherwise clear, but vacuolated. The nucleus is large, rounded and vesicular. A clear halo is often seen surrounding it.



Pig. 1.

Photomicrograph showing a glomerulus of a human kidney with afferent arteriole, juxtaglomerular corpuscle (xx) and adjacent macula densa of the distal convoluted tubule (x). Eulargement 360 X.

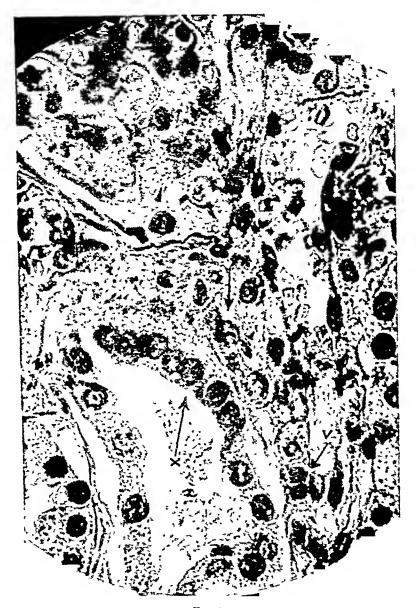


Fig 2

Photomicrograph of macula densa (τ) and juxtaglomerular corpuscle (τx) in a human kidney. Note the halo around some of the nuclei in the corpuscle and the vacuolization of the cytoplasm. Several intercellular fibrils are also visible. (τ) indicates smooth muscle cells of the arteriolar media. Enlargement 600 \times .

Taugential sections of mouse, rat, rabbit and cat kidneys, starting from the cortical surface and proceeding toward the medulla show that the juxtaglomerular corpuscles are absent in the superficial layers of the cortex corticis, but increase markedly toward the middle part of the cortex and decrease again toward the cortico-medullary junction. They seem to be intrinsic characteristic structures of the normal, functioning mammalian kidney. The distribution of the macula densa in the kidney parallels that of the juxtaglomerular corpuscles.

It is important that the tissues be fixed while very fresh, preferably in Bouin's or Zenker's solution. They may be embedded in paraffin in the usual way and sectioned serially, if possible at 4 to 6 microns. Preparations stained with hematoxylin-cosin show the structures clearly, but do not give adequate cytologic details. Masson's trichrome stain is more satisfactory and Mallory's phosphotungstic acid-hematoxylin reveals good nuclear detail. Cytoplasmic vacuolization is clearly visible with this stain and with Mallory's anilin blue connective tissue stain. Intercellular fibrils are well brought out with Masson's stain or better with silver stains.

No definite suggestion as to the nature, the biological or physiological function of these cells can yet be given. Their intimate relationship with the macula densa is noteworthy and may suggest a physio-biologic interrelation between these two structures.

11411

Influence of Neoprontosil on Migration of Blood Leucocytes in Tissue Cultures.*

AUSTIN F. HENSCHEL. (Introduced by Joseph T. King.)
From the Department of Physiology, The University of Minnesota, Minneapolis.

Sulfanilamide and Neoprontosil have been reported to stimulate phagocytosis of bacteria by leucocytes in vitro. Finkelstein and Birkeland¹ found that in the presence of sulfanilamide and Prontosil (Neoprontosil?) the number of guinea pig leucocytes taking up bacteria and the number of bacteria engulfed per leucocyte was markedly increased. Fresh plasma or serum appeared to be neces-

^{*}The Neoprontosil was furnished by the Department of Medical Research, Winthrop Chemical Co., Inc.

¹ Finkelstein, R., and Birkeland, J. Y., Science, 1938, 87, 441.

sary in order to obtain greater phagocytic activity in the presence of

the drugs.

Gay, et al.,² however, found no definite difference in the *in vitro* phagocytosis of streptococci by exudate cells from sulfanilamide-treated rabbits as compared with normal rabbits. Streptococci that were treated with sulfanilamide were more readily phagocytized than non-treated streptococci.

Tunnicliff³ observed that both sulfanilamide and Neoprontosil increased the phagocytosis of streptococci by blood leucocytes. She states that "By comparing the amount of phagocytosis in the leukocytes suspended in salt solution with that in leukocytes suspended in dilute prontosil-soluble the stimulating action of prontosil-soluble was observed to be on the leukocytes."

When Neoprontosil in 1:1000 concentration was added to whole blood with bacteria, the number of cocci ingested per leucocyte was doubled. Serum from mice receiving molar equivalents of sulfanilamide and Neoprontosil increased the phagocytic activity of normal leucocytes to the same degree.

King⁴ demonstrated that sulfanilamide 1:1000 stimulated the rate of migration of rabbit leucocytes in sterile tissue cultures. After 24 hours of incubation the average migration rims were 18% wider than in the controls. The absolute difference was 5.8 times the standard error of the difference.

The influence of Neoprontosil (the disodium salt of 4' sulfamido-phenyl-2-azo-7 acetylamino-1 hydroxynaphthalene-3, 6 disulfonic acid) on the migration rate of blood leucocytes in sterile tissue cultures was studied.

The routine culture methods used were described by King.^{5, 6} To obtain the buffy coat, 15 cc of rabbit blood were drawn into a 50 cc centrifuge tube containing sufficient heparin to prevent clotting. The blood was centrifuged at high speed for 15 minutes and the plasma removed. The tube was recorked with a fresh sterile cork and recentrifuged for one-half hour. If the tubes are handled carefully, the buffy coat can at the end of the second centrifugation be removed as a solid placque. After washing the buffy coat with Tyrode to remove the adhering red cells, it was fragmented and covered with Tyrode. Buffy coat fragments are extremely fragile. If allowed to

² Gay, F. P., Clark, A. R., Street, J. A., and Miles, D. W., J. Exp. Med., 1939, 69, 607.

³ Tunnicliff, R., J. Inf. Disease, 1939, 64, 59.

⁴ King, J. T., Am. J. Physiol., 1938, 123, 119.

⁵ King, J. T., Arch. f. Exp. Zellforsch., 1930, 9, 341.

⁶ King, J. T., Arch. f. Exp. Zellforsch., 1931, 10, 467.

remain in a fluid medium for more than one hour or handled roughly, they disintegrate rapidly.

The fragments, 2 to 3 mm in diameter, were carefully chosen in pairs according to size, shape and general texture. One fragment from each pair was used as the control, the other fragment as the experimental tissue. In this way each control culture has a visually identical experimental culture. Each series contained 30 cultures made from one animal

The cultures were planted in moist chambers (Maximow technic) and incubated as lying drops in a special down-draft incubator described by King.⁷

The fragments were planted in one part autogenous heparinized plasma and 3 parts of an autogenous rabbit serum extract of 6-day chick embryos. Sufficient Neoprontosil was added to the serum extract to make a 1:1000 concentration in the final culture medium.

Observations were made on the living cultures at the end of 24 hours of incubation. The maximum migration rim was measured with a 16 mm objective and a $6\times$ ocular with an eyepiece micrometer (114 units = 1 mm).

TABLE 1.

	No. of				•	
No. of series	cultures per series	Avg control	Avg experimental	Absolute difference	S.E. of difference	70 increase
16	30	220	242.8	22.8	7.48	10.3

The experimental data are shown in Table I. From the results it appears that Neoprontosil 1:1000 increases the rate at which rabbit blood leucocytes migrate in tissue culture media. The absolute difference in the width of the migration rims between the experimental and control cultures is 22.8 and the standard error of the difference is 7.48.

Whether there is any significant correlation between the increased migration and the increased phagocytosis by lencocytes in vitro is not clear at present.

The possibility that the Neoprontosil might be partially split into sulfanilamide in tissue culture media and exert its stimulating effect as such must be considered. No experimental evidence is available on this point.

Conclusion. In tissue culture media composed entirely of animal body fluids Neoprontosil 1:1000 stimulates the rate of rabbit blood leucocyte migration.

⁷ King, J. T., Arch. f. Exp. Zellforsch., 1937, 20, 208.

11412 P

Experimental Coronary Occlusion and Myocardial Fibrosis.

MARK E. MAUN. (Introduced by W. O. Nelson.)

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Detroit, Mich.

Colloidal solution of aluminum hydroxide was observed by Beland, Moe, and Visscher¹ to produce rapid stoppage of the heart when the aluminum was perfused into the coronary arteries in a heart-lung preparation. They believed that coronary insufficiency resulted from multiple capillary emboli due to the injection of the aluminum hydroxide. A 2% aluminum hydroxide solution was also injected into the right side of the heart in a similar preparation. In this instance the lungs apparently filtered out sufficient aluminum so that no deleterious effect was noted on the coronary circulation. Irwin² studied the effect of aluminum solutions injected intravenously in experimental animals. He found that if sufficient quantities of the metal were injected the animal died due to pulmonary emboli. Nodular fibrotic areas were noted in the lungs of rabbits following experimental inhalation of aluminum dust;³ the tissue response was that of a foreign body reaction.

In the present study the sternums of 4 control rabbits were removed so that aluminum hydroxide could be injected directly into the cavity of the beating left ventricle. Large doses of the material were injected until the animals died. No gross changes were apparent in the heart or other organs. Microscopic study of various sections of the heart muscle with the hematoxylin and eosin stain reveals numerous small arterioles to be filled with bluish-purple masses which partially or completely occluded the smaller arterioles. In those vessels only partially occluded with the colloidal material the aluminum was found to adhere to the wall of the vessel.

In order to prove that the suspected masses were aluminum the sections were stained for aluminum using a specific stain. The aluminum particles were noted to be cherry-red in color by this method. In some instances the very small aggregates of the material seen in small capillaries with the hematoxylin and eosin stain failed to take the red color with a specific stain (Aurine). This

¹ Beland, I. J., Moe, G., and Visscher, M. D., PROC. Soc. EXP. Bio . AND Med., 1938, 39, 145.

² Personal communication.

³ Denny, J. J., Robson, M. B., and Irwin, D. A., Canad. Med. Assn. J., 1937, 37, 1.

remain in a fluid medium for more than one hour or handled roughly, they disintegrate rapidly.

The fragments, 2 to 3 mm in diameter, were carefully chosen in pairs according to size, shape and general texture. One fragment from each pair was used as the control, the other fragment as the experimental tissue. In this way each control culture has a visually identical experimental culture. Each series contained 30 cultures made from one animal.

The cultures were planted in moist chambers (Maximow technic) and incubated as lying drops in a special down-draft incubator described by King.7

The fragments were planted in one part autogenous heparinized plasma and 3 parts of an antogenous rabbit serum extract of 6-day chick embryos. Sufficient Neoprontosil was added to the serum extract to make a 1:1000 concentration in the final culture medium.

Observations were made on the living cultures at the end of 24 hours of incubation. The maximum migration rim was measured with a 16 mm objective and a 6× ocular with an eyepiece micrometer (114 units = 1 nm).

TABLE L

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Whether there is any significant correlation between the increased migration and the increased phagocytosis by leucocytes in vitro is not clear at present.

The possibility that the Neoprontosil might be partially split into sulfanilamide in tissue culture media and exert its stimulating effect as such must be considered. No experimental evidence is available on this point.

Conclusion. In tissue culture media composed entirely of animal body fluids Neoprontosil 1:1000 stimulates the rate of rabbit blood

leucocyte migration.

⁷ King, J. T., Arch. f. Exp. Zellforsch., 1937, 20, 208.

11413 P

Liberation of a Histamine-Like Substance on Stimulation of Sympathetic Nerves.

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College of Medicine, University of Illinois, Chicago.

Several attempts have been made to find a nervous mechanism for the release of histamine from body tissues, 1,2 but thus far the only evidence that such may exist has been indirect. The present study deals with the release of histamine by the skin upon stimulation of sympathetic nerves. The epidermal layer of the skin contains relatively large quantities of histamine (24 y/g).

Experiments were carried out on rabbits, unanesthetized or anesthetized lightly with urethane or ether. The cervical sympathetic trunk and its superior ganglion were prepared for stimulation with bipolar electrodes (4 to 60 sec, primary 3 volts, secondary coil at 10 cm). Blood samples obtained from the great auricular vein by venipuncture before and after nerve stimulation were compared for their ability to contract an isolated segment of guinea pig ileum according to the method of Schultz and Dale. Atropinized Ringer-Locke solution was used in the muscle bath in every case. Standard histamine solutions were used for comparison of contractions.

In early experiments the blood samples were allowed to clot; the serum obtained was diluted immediately with Ringer's solution or buffer solution of pH 7.15 and tested on the guinea pig ileum. In these experiments (10 animals) serum obtained 15 to 60 seconds following nerve stimulation almost invariably produced a greater contraction of the guinea pig ileum than the control serum (15 to 50% greater). This difference was present after and often increased by heating the diluted sera in a water bath at 60° to 70°C for 30 minutes, provided the pH of the sera was not above 7.15. The entire contractor effect of both control and stimulation sera could be abolished by previous addition of 0.5 γ of thymoxyethyldiethylamine to the muscle bath.

Rabbit blood has already a high content of histamine which is almost entirely stored in the cellular elements (platelets, Minard⁴),

^{*} Partially aided by a grant from Abbott Laboratories.

¹ MacGregor, R. R., and Peat, S., J. Physiol., 1931, 71, 31.

² Bulbring, E., and Burn, J. H., J. Physiol., 1935, 83, 483.

³ Lewis, T., and Marvin, H. M., Heart, 1927, 14, 27.

⁴ Minard, D., Am. J. Physiol., 1937, 119, 375.

may be due to the small quantities of the aluminum present. After some experience the small aluminum thrombi can be detected with little difficulty, using hematoxylin and eosin stain following formalin fixation.

Thirty rahbits were given repeated intraventricular injections of aluminum hydroxide in non-fatal amounts. In animals weighing from 1800 to 2500 g, 1 cc of the 2% solution could be safely given. Twenty-two of the rahbits lived and were sacrificed from one to 6 weeks following repeated injections of the aluminum into the left ventricle while others died immediately following such injections.

Gross examination of the hearts of the 22 animals which survived revealed fibrotic patches in some. A few yellowish areas measuring one millimeter in diameter could be observed in others. Microscopic examination of the heart muscle of these animals revealed numerous small fibrotic patches scattered particularly throughout the left ventricle. These scarred areas are similar to those seen in human cases that are associated with coronary sclerosis. In a few of the sections a chronic inflammatory reaction was the most pronounced change present.

A number of the hearts showed small areas of infarction, particularly in the lower third of the septum and near the tip of the left ventricle. Some of the small arterioles seen in the adjacent myocardium were partially or completely occluded by aluminum thrombi. The cardiac muscle adjacent to the infarcts was partially replaced by actively proliferating fibroblasts and numerous inflammatory cells. The latter consisted of lymphocytes, eosinophils and numerous macrophages.

A more chronic proliferative lesion was noted in some areas of the heart in which large amounts of aluminum were present. In addition to the partial occlusion of the vessels some of the material apparently incited a foreign body reaction. The macrophages in those sections contained numerous particles of aluminum. A few giant cells of the foreign body type were also seen in the nearby tissue. The marked proliferative response of the fibroblasts in some instances produced a picture similar to true Aschoff nodules. Changes in the vessels were not especially frequent; the change most commonly observed was some intimal proliferation and an increased thickness of the vessel wall.

Summary. It is possible to produce coronary occlusion and myocardial fibrosis experimentally in the rabbit by means of injection of colloidal aluminum hydroxide into the left ventricle. This method may be useful in the experimental study of cardiac hypertrophy and coronary disease.

The identification of the contractor substance is aided by the use of thymoxyethyldiethylamine. This drug is specific in counteracting the effect of histamine on the guinea pig ileum. It has no comparable effect upon contractions produced by KCl. NaHCO₂, acetylcholine, or the contraction produced in rare instances by adrenaline. Acetylcholine produces no contraction of an atropinized muscle. The slight changes in pH of the blood following nerve stimulation were not sufficient to affect the activity of the muscle strip and most sera were diluted with a buffer solution. On the other hand, the contractor substance obtained in the blood from the rabbit's ear following nerve stimulation was heat stable, active in an atropinized bath, but inactive after addition of thymoxyethyldiethylamine. In these respects it is "histamine-like". The source of this substance in the rabbit's ear has not been determined.

We wish to acknowledge the assistance of Mr. C. J. Loechl in these studies.

11414 P

Thymoxyethyldiethylamine Antagonism to Circulatory Effects of Histamine in Anesthetized and Nonanesthetized Dogs.

DAVID MINARD AND SOL R. ROSENTHAL.

From the Departments of Phylodical, and of Pathology, Barbertology, and Public Health, College of Medicine of the University of Il no s, Climage.

Although thymoxyethyldiethylamine (Thym.) has been found to antagonize histamine (Hi) effects on isolated smooth muscle¹⁴ and to exert a protective action in guinea pigs against anaphylactic and Hi shock.^{2, 2, 3} little work has been done regarding its action in carnivorous animals in which the circulatory effects of Hi are most striking. We have undertaken to investigate Thym. antagonism to the hypotension and hemoconcentration resulting from Hi administration in anesthetized and nonanesthetized dogs, and to observe the effects of Thym. alone

¹ Re-artial, S. R., and Minard, D. J. Exp. Med., 1979, 70, 415.

¹ Bover, D., and Stanb. A. M., C. E. Sec. Bert., 1937, 124, 547

² Stanb, A., and Borer, D. C. R. Sec. Bief., 1937, 125, 818.

³ Stanb. A. M., Ann. Inst. Pastenn. 1939, 63, 460, 485.

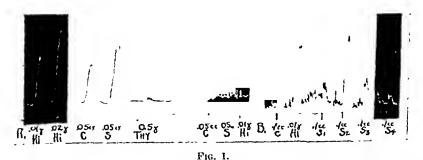
⁴ Resential, S. R., and Minard, D., J. Erg., Med., 1939, 70, 415.

Resenthal S. R. and Brown, M. L. J. Immuno', in press.

but is liberated into the serum after clotting. Since only that histamine found free in the blood plasma is active in vivo, an attempt was made to determine whether there was an increase in plasma histamine after nerve stimulation (5 animals). Whole blood (0.1 to 0.2 cc) was drawn directly into a syringe containing an equal quantity of heparinized Ringer's solution. These were drawn, mixed, and added at once to the muscle bath. It was found that control samples of unclotted blood contained no demonstrable contractor substance. However, following nerve stimulation, ability to contract the muscle appeared in ½ to 3 minutes and was usually indetectable again after 8 to 15 minutes. This property of the whole blood could be abolished by previous addition of thymoxyethyl-diethylamine to the muscle bath. The experiment could be repeated more than once using the same rabbit car.

Two experiments with cats, testing blood serum, gave results similar to those in the rabbit. Blood was drawn from the external jugular vein following stimulation of the superior cervical sympathetic ganglion.

Adrenalin which inhibits to some extent the muscle contraction produced by histamine is liberated on stimulation of sympathetic nerves to the rabbit's ear. Frequently, in our own experiments, blood drawn immediately following stimulation inhibited the effect of standard histantine solutions added to the bath more than did control samples. Our results may have been made less apparent by this antagonism.



A. Effect of thymoxyethyldiethylamine on C, control scrum, on S, scrum obtained after stimulation of the superior ganglion (4 sec), and on Hi, standard historius.

B. Effect on the ileum of C, control heparinized, whole blood; S_1 , obtained immediately after stimulation of gauglion (10 sec); S_2 , 2 min after stimulation; S_3 , 4 min after stimulation; S_4 , 13 min after stimulation.

⁵ Code, C. F., J. Physiol., 1937, 90, 349.

⁶ Gaddum, J. H., Jang, C. S., and Kwiatkowski, H., J. Physiol., 1939, 96, 104.

				TABLE II.					
Hemoconcentration	After	$_{ m Hi}$	in	Nonanesthetized	Dogs	With	and	Without	Thym.
				Treatment.					

		emoconc., avg itial level)	Recovery time, avg (To 10% of initial level)		
No. of expts.	Histamine	Thym. + Histamine	Histamine	Thym. + Histamine	
9 7	26.5	11.8	> 110 min	30 min	

reduction in both the degree and duration of hemoconcentration following histamine in animals receiving Thym. On the other hand, the blood pressure effects of Hi (1 mg/kg) were not appreciably altered by Thym. treatment.

Discussion. Staub³ failed to observe a decrease in the depressor response to Hi in chloralosed dogs after intravenous Thym. injection, probably because the Hi doses used (.02 mg/kg) were beyond the effective range of Thym. However, we can confirm in dogs this author's observations in guinea pigs on Thym. antagonism to bronchoconstrictor effects of Hi.

Insufficient experimental work precludes any postulation regarding the mechanism of Thym. antagonism to the depressor effects of small Hi doses. However, it seems unlikely that Hi vasodilatation is involved; more probably Thym. action is limited to abolishing venoconstrictor effects of Hi.

Whether the marked hemoconcentration observed in nonanesthetized dogs after Hi represents solely a loss of plasma volume or whether an influx of red cells from a reservoir such as the spleen is an important factor has not been investigated. Hence, it is premature to suggest that Thym. may reduce loss of plasma volume in histamine shock.

Summary. Thymoxyethyldiethylamine administered subcutaneously to (a) anesthetized and (b) nonanesthetized dogs reduces or abolishes the depressor effects of small histamine (Hi) doses and prevents the bronchial constrictor action of large Hi doses in (a) and reduces the hemoconcentration following large Hi doses in (b), but in neither (a) nor (b) are the depressor effects of large Hi doses appreciably altered.

In the first series of experiments, dogs under light amytal anesthesia (60 mg/kg intraperitoneally) were used. Mercury manometer recording of carotid pressure, tracheal cannulation with pneumometer recording of respiration, and cannulation of the femoral vein for injection completed the preparation.

Four dogs served to establish the effects of small $(1-10 \ \gamma)$ and large $(.25-1.0 \ \text{mg/kg})$ doses of Hi injected intravenously. Accompanying the blood pressure fall after large Hi doses, signs of severe respiratory obstruction were observed and taken to indicate bronchial constriction.

In 10 dogs prepared as above, depressor effects of 1-10 γ doses of Hi were studied before and after subcutaneous administration of 40 mg/kg Thym. After Thym, the depressor effects of these Hi doses were markedly decreased. Acetylcholine responses were affected but slightly, thus indicating a specificity of Thym, action similar to that observed on isolated smooth muscle.

TABLE I.

Depressor Responses to Hi and Acetylcholine Before and After Thym.

	Before Thym.	After Thym.
Hi 4 γ	39 mm Hg 40	10 mm Hg 30

After large Hi doses, the drug had no apparent effect either on the degree of blood pressure fall or recovery rate; however, indications of bronchial constriction were no longer evident.

In the second series of experiments, using nonanesthetized dogs, arterial pressure readings from the femoral artery exposed under local anesthesia were obtained with a needle and anaeroid manometer, a spinal fluid trap being interposed. Blood samples were drawn and injections were made into the femoral vein. Hemoglobin concentrations were determined by the Sanford-Sheard photelometer.

The effects of subcutaneous administration of 40 mg/kg Thymon pulse rate, blood pressure, and hemoconcentration were observed in 5 animals. The pulse and blood pressure uniformly showed a rise in 5-10 minutes after injection, reached a peak in 30-45 minutes, and gradually declined to normal. The hemoglobin level usually showed a transitory rise of less than 10%.

The effects of Hi (1 mg/kg intravenously) were studied in 16 experiments on 9 dogs with and without previous Thym. administration. The results, summarized in Table II, indicate a significant

⁶ Sanford, A. H., Sheard, C., and Osterberg, A. E., Am. J. Chn. Path., 1933, 3, 405.

TABLE I.

		Avg urine volume in cc					
Treatment	No. of Exp.	9-1	1-5	5 -9	9-1	1-9	24 hr Total
Control	15	980	1410	950	660	975	4975
1 cc 0.2% ZnAc	4	1110	1230	870	650	1120	4980
1 '' pitressin	7	290	380	500	540	980	2690
1 ' 0.2% ZuAc } 1 ' pitressin }	8	260	150	190	140	690	1430
Pitressin-in-oil	3						
Control day		970	1250	970	640	1100	4930
First ",*		445	320	50	120	200	1135
Second ''		0	320	220	80	490	1110
Third ''		200	770	870	290	780	2910
Fourth ''		860	1030	510	350	1000	3750
Fifth ''		700	1550	1160	460	980	4850

^{*}Pitressin-in-oil, 1.3 cc (20 units), injected intramuscularly at 9 A.M., on the first day only.

most striking during the first 4 hours, with some reduction in urine volume for as long as 12 hours after injection. The administration of a mixture of 1.0 cc of pitressin and 1.0 cc of 0.2% zinc acetate solution resulted in prolongation and intensification of the pitressin effect during the period from 4 to 16 hours after injection. The effect of both the aqueous pitressin and the zinc-pitressin mixture was dissipated within 24 hours.

The effect of the administration of a preparation of pitressin suspended in peanut oil was more striking. The results of 3 experiments are summarized in Table I. A single intramuscular injection of 1.3 cc (20 units) of pitressin-in-oil resulted in a maximal pitressin effect of fully 48 hours' duration, as contrasted with a 4- to 8-hour maximum effect in the case of a similar dose in aqueous solution and a 16-hour maximum effect when given in an aqueous solution of a mixture of pitressin and zinc acetate. With the oil preparations there was some effect evident for as long as 72 hours.

These observations suggest that when pitressin is given alone, in watery solutions, it is rapidly absorbed and its full effect lost through destruction or excretion of the active principle. The data presented in this communication are compatible with the view that absorption of the pitressin is delayed when given in the presence of zinc acetate, or in oil.

Summary. The presence of 0.1% zinc acetate prolonged and intensified the effect of aqueous solutions of pitressin in reducing the water exchange in experimental diabetes insipidus. The use of a preparation of pitressin suspended in oil resulted in a much more marked prolongation and intensification of the pitressin effect.

11415 P

Zine Salts and Oil in Prolongation of Therapeutic Effect of Pitressin in Experimental Diabetes Insipidus.

D. J. STEPHENS. (Introduced by W. S. McCann.)

From the Department of Medicine, University of Rochester School of Medicine, and the Medical Clinic, Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.

It has been shown by previous investigators that the addition of zinc salts increases the activity of hypophyseal gonadotropic extracts^{1, 2} and prolongs the hypoglycenic effect of insulin.³ Boyd and Clark¹ have reported that the addition of zinc salts to posterior pituitary extracts prolongs the retention of water taken up by frogs. Keeney, Pierce and Gay⁵ have shown that the effect of epinephrine is greatly prolonged when it is given suspended in oil. The present communication is a preliminary report of an investigation of the effect of zinc salts and of oil on the action of pitressin in experimental diabetes insipidus.

A dog with experimental diabetes insipidus⁶ was loaned to the author for this study by Dr. Roland Bellows of the Department of of Neurosurgery. The animal was fed once daily at 9 A.M.; liberal amounts of drinking water were available at all times. The urine was collected in fractional samples every 4 hours, with the exception of the period between 1 A.M. and 9 A.M., when a single 8-hour specimen was collected. The urine volume and specific gravity were accurately measured.

Test substances were injected in a single dose at 9 A.M. The results of the subcutaneous injection of 1 cc of 0.2% zinc acetate solution, of 1 cc of pitressin* and of a mixture of 1 cc of pitressin and 1 cc of 0.2% zinc acetate solution arc shown in Table I. The subcutaneous administration of zinc acetate alone had no effect. The administration of a single dose of 20 units of pitressin resulted in a moderate reduction of the urine volume. The pitressin effect was

¹ Fevold, H. L., Hisaw, P. L., and Greep, R., Am. J. Physiol., 1936, 117, 68.

² Saunders, F. J., and Cole, H. H., Proc. Soc. Exp. Biol. and Med., 1936, 33, 505.

³ Scott, D. A., and Fisher, A. M., J. Phaim. and Exp. Therap., 1935, 55, 206.

⁴ Boyd, E. M., and Clark, K. J., Am. J. Med. Sci., 1939, 198, 171.

⁵ Keeney, E. L., Pierce, J. A., and Gay, L. N., Arch. Int. Med., 1939, 63, 119.

⁶ Bellows, R. T., and VanWagenen, W. P., J. Nerv. and Mental Dis., 1938, 88, 417.

[•] The pitressin and the pitressin in oil used in this study were generously supplied by Dr. E. A. Sharp of the Parke Davis Co.

DAILY DOSES FOR 4 WEEKS

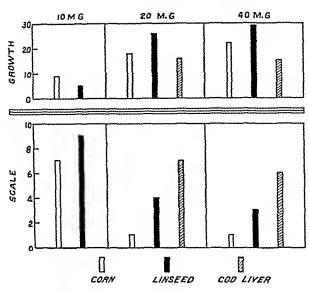
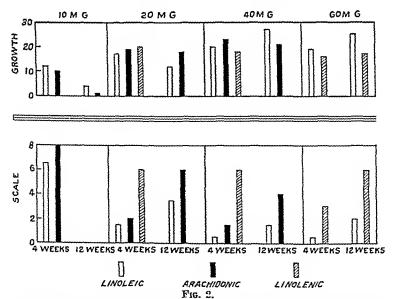


Fig. 1.

Growth and skin cure during 4 weeks of feeding oils at 3 levels. Poor skin cure is indicated by high column for scale.

DAILY DOSES FOR 4 WEEKS



Growth and skin cures with 3 pure fatty acids. The doses were fed daily for 4 weeks and stopped, while growth and skin records were continued 8 more weeks to show storage effects.

11416

Comparative Curative Values of Unsaturated Fatty Acids in Fat Deficiency.*

G. O. BURR, J. B. BROWN, J. P. KASS AND W. O. LUNDBERG. From the Department of Botany, University of Minnesota.

Some time ago¹ we called attention to the fact that cod liver oil fed at 2-5 drop levels daily caused renewed growth of fat-deficient rats but did not clear the scaly skin. In recent years we have had occasion to test numerous oils and pure fatty acids for these effects. Some of the results are reported in this paper.

Fat deficiency in white rats was produced with the diet 550-B used by Burr and Burr² consisting of casein, sucrose and salts supplemented with whole dried yeast (Northwestern) and the unsaponifiable fractions of cod liver oil and wheat germ oil. When a growth platean is reached and the skin has become scaly the animals are used for a study of the curative effects of the oils and fatty acids. The results are recorded as gains in weight in a given time and in apparent change in skin condition. An arbitrary rating of scale on the feet, scale on the tail and dandruff in the hair, ranging from 0 to 3 (most severe) is summed to give a maximum scaly condition of 9. Three to 6 rats are used in each group and the results have been reneated several times.

During a search of corn oil, linseed oil and codliver oil for isomeric fatty acids the oils were fed at low levels and the responses noted at the end of 4 weeks. The results are recorded in Fig. 1. Ten mg daily of corn or linseed oil is not sufficient to produce marked improvement. At the 20 and 40 mg levels, however, all 3 oils give good growth responses. On the other hand, corn oil is the only one which produces a complete skin cure in this period of time. These differences can be explained if we assume that linoleic acid is responsible for the clearing of the skin, while linolenic and cod liver oil acids have little or no such effect. Corn oil (Mazola) has over 60% linoleic acid. Linseed oil probably has 30% linoleic and 40% linolenic acids.

^{*}Assistance in the preparation of these materials was furnished by the personnel of Works Projects Administration, Official Project No. 65-1-71-140, Subproject No. 325.

¹ Burr, G. O., Burr, M. M., and Biown, W. R., Proc. Soc. Exp. Biol. and Med., 1931, 28, 905.

² Burr, G. O., and Burr, M. M., J. Biol. Chem., 1929, 82, 345.

DAILY DOSES FOR 4 WEEKS

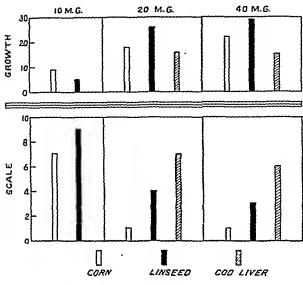
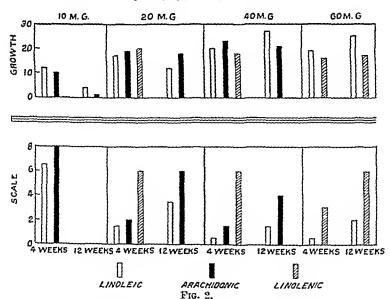


Fig. 1.

Growth and skin cure during 4 weeks of feeding oils at 3 levels. Poor skin cure is indicated by high column for scale.

DAILY DOSES FOR 4 WEEKS



Growth and skin cures with 3 pure fatty acids. The doses were fed daily for 4 weeks and stopped, while growth and skin records were continued 8 more weeks to show storage effects.

In Fig. 2 are summarized the results of feeding linoleic, linolenic and arachidonic acid. In these experiments the doses were fed for only 4 weeks and the observations continued for 8 more weeks to measure the storage effects. It is clear that linolenic acid gives good growth responses but has little effect on the skin. Arachidonic and linoleic acids are similar in their effects. When fed at these low levels we do not find the superior action of arachidonic acid cited by Turpeinen. When fed at a 40 mg level there is a storage effect of linoleic acid which makes those animals superior in both weight and skin quality after 8 weeks on the fat-deficient ration.

These findings are of particular interest at this time in the study of the relation of unsaturated acids to vitamin B, deficiency. It has been observed by numerous workers that certain oils cure the severe acrodynia accompanying B_n deficiency. Schneider, Ascham, Platz and Steenbock⁴ recently summarized their findings on the antiacrodynic potency of foods. Examination of their Table I shows that cod liver oil is very poor and the linolenic acid of linseed oil is ineffective. Those oils highest in linoleic acid are most effective (corn oil and wheat germ oil). Salmon⁵ recently reported that methyl linolate was much more effective than methyl linolenate in curing the skin of B_n deficient rats.

Conclusions. Unsaturated fatty acids (linoleic, linolenic, arachidonic and cod liver oil acids) show differences in growth and skin effects. They should no longer be treated as an interchangeable group but should be used individually in nutrition studies.

³ Turpeinen, O., J. Nutr., 1938, 15, 351.

⁴ Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., J. Nutr., 1939, 18, 99.

⁵ Salmon, W. D., Proc. Soc. Biol. Chem., 1940, 34, 83.

11417

Pyruvic Acid in Blood and Cerebrospinal Fluid.*

Ernest Bueding and Herman Wortis. (Introduced by N. Jolliffe.)

From the Psychiatric Division, the Medical Service of the Psychiatric Division, Bellevue Hospital, New York City, and the Department of Psychiatry. New York University College of Medicine.

It has been shown that cocarboxylase (the diphosphoric ester of vitamin B₁) is necessary for the normal catabolism of pyruvic acid. In vitamin B₁ deficiency (Oriental Beri-beri) pyruvic acid accumulates in the body fluids. Methods previously used in the determination are not satisfactory since a significant decrease in blood pyruvate occurs if the blood be allowed to stand at room temperature for even one minute prior to precipitation. This disappearance does not occur if monoiodoacetate in a concentration of 0.2% is used as a stabilizing medium. Utilizing this finding, we have recently described a method for the stabilization and determination of pyruvic acid in the blood.

The present study was undertaken in order (1) to determine the degree of stability of pyruvic acid in the spinal fluid, (2) to compare the levels of pyruvic acid in the blood and cerebrospinal fluid, (3) to make certain preliminary observations concerning the value of pyruvic acid determinations for the diagnosis of vitamin B₁ deficiency.

Method. The method for blood determinations was also used for the spinal fluid, except that the stabilizing medium (monoiodoacetate) was found to be unnecessary.

Stability of Pyruvic Acid in the Spinal Fluid. In contrast to our findings in the blood, the pyruvic acid content of the spinal fluid remains constant even if the sample be allowed to stand at room temperature for one hour prior to precipitation. Additional evidence of the stability of pyruvic acid in the spinal fluid was obtained in the following way: Five cc samples of cerebrospinal fluid were caught in test tubes containing 25 mg of sodium monoiodoacetate, and other samples of the same spinal fluid were allowed to stand at room temperature for 60 minutes prior to precipitation without the use of the

^{*} This work was aided by a grant from Child Neurology Research (Friedsam Foundation).

¹ Banga, I. L., Ochoa, S., and Peters, R. A., Biochem. J., 1939, 33, 1109.

² Platt, B. S., and Lu, G. D., Quart. J. Med., 1936, 5, 355.

³ Bueding, E., and Wortis, H., J. Biol. Chem., 1949, 133, 585.

	TABLE	I.		
Stability of	Pyruvate in	the	Spinal	Fluid.

Spinal fluid sample No.	Precipitated after 3 min mg% pyruvic acid	25 mg CH ₂ ICOONa ndded. Precipitated after 3 min mg% pyruvic acid	No CH2ICOONa added. Precipitated after 60 min mg% pyruvic acid
1	0,90		0.88
2	0.93		0.93
3		0.86	0.86
4		1.04	1,05
5		0.83	0,83
6	1.12	1,09	1.10
7	0.78		0.79
8*		2.00	2.18
9		2.40	2.31

^{*8} and 9 were eases of pneumococcus meningitis.

stabilizing medium. Identical values were obtained in both samples (Table I).

Pyracic Acid in the Blood and Cerebrospinal Fluid. Simultaneous samples of blood and cerebrospinal fluid were obtained on 67 patients with various neuropsychiatric and medical disorders. The subjects were fasting and at rest in bed. All determinations were done in duplicate.

The content of blood pyruvic acid in 60 normal subjects was previously found to vary from 0.77-1.16 mg %. We have, therefore, arbitrarily decided to consider as abnormally high those cases with blood pyruvate levels of 1.30 mg % or above.

Considered in this fashion, 51 of our 67 cases had normal values for pyruvic acid in the blood, and 16 of our cases showed elevated figures. The relationship of spinal fluid pyruvate to that of the blood in these 2 groups is seen in Table II.

Preliminary Observations Concerning the Value of Pyruvic Acid Determinations for the Diagnosis of Vitamin B₁ Deficiency. The 51 cases with normal blood levels formed the following diagnostically labelled groups: (a) chronic alcoholism without clinical evidence of vitamin B₁ deficiency (peripheral neuropathy or beri-beri), 15 cases;

TABLE II.
Relationship of Blood to Spinal Fluid Pyruvate.

		Mg% pyruvie neid				
Group	No. of cases	Blood	C.S.F.	% C.S.F./ blood		
1	51	0.79-1.30 (Avg 1.03)	0.42-1.52 (Avg 0.84)	43-118* (Avg 82)		
2	16	1.41-2.41 (Avg 1.82)	1.03-2.40 (Avg 1.77)	67-170* (Avg 97)		

^{*} In only 6 cases (2 in Group 1 and 4 in Group 2) did the % C.S.F./Blood fall outside the range 70-120%.

(b) Schizophrenia, 10 cases: (c) behavior problems in children, 6 cases: (d) mental deficiency in children, 5 cases: (e) pneumonia, 5 cases: (f) psychopathic personality, 2 cases: (g) hyperthyroidism, 2 cases: (h) senile psychosis, 2 cases: (i) one case each of arsenical poisoning, reactive depression, paroxysmal convulsive disorder of unknown etiology and herpes zoster.

The 16 cases with high blood levels formed these diagnostically labelled groups: (a) chronic alcoholism with acute peripheral neuropathy. 12 cases: (b) beri-beri. 1 case: (c) pneumococcus meningitis. 2 cases: (d) pneumonia with prolonged temperature elevation. 1 case.

Of the 16 cases with elevated pyruvic acid levels, it is noteworthy that 13 (12 of acute peripheral neuropathy and one of beri-beri) occurred in clinical syndromes which are known to be the result of vitamin B, deficiency. As a matter of fact, Platt and Lu have previously described elevations of blood pyruvate in cases of fulminating beri-beri.2.4 We have criticized the limitations of their method in previous communications.^{3, 5} In the 3 remaining cases with elevated blood pyruvate, the increase in total metabolism, as a result of prolonged fever with resultant depletion of vitamin B₁, may have contributed to the high values obtained. It should, however, be particularly noted that none of these cases showed evidences of acute peripheral neuropathy. It may be that the metabolic disturbance must exist for some time before clinical evidences of vitamin B, deficiency are apparent. In addition, further work may indicate that elevations in blood pyruvate are related to conditions other than avitaminosis B.

Nonetheless, our results take on added interest if we reexamine our cases of chronic alcoholism without clinical evidences of vitamin B₁ deficiency. These 15 cases are subdivided as follows: (a) chronic alcoholism with old or treated peripheral neuropathy. 8 cases: (b) chronic alcoholism without evidences of involvement of the central or peripheral nervous systems. 4 cases: (c) chronic alcoholism with nicotinic acid deficiency. 2 cases: (d) chronic alcoholism with brain laceration. 1 case. The pyruvic acid levels were normal in every case. On the other hand our cases of chronic alcoholism with clinical evidences of vitamin B₁ deficiency showed an elevation of blood pyruvate in every instance.

Summary. 1. The stability of pyruvic acid in the spinal fluid is

⁴ Lu. G. D., Biochem. J., 1939, 33, 774.

⁵ Wortis, H., Bueding, E., and Wilson, W., PEOC. Soc. EXP. BIOL. AND MED., 1940, 43, 279.

described. 2 The relationship of blood to spinal fluid pyruvate is reported. The amount found in the cerebrospinal fluid is usually 70-120% of that found in a blood sample taken simultaneously. 3. Of the 16 cases with elevated blood pyruvate, 13 occurred in cases of known vitamin B_t deficiency. These latter constituted the only cases in the entire study with definite clinical evidences of vitamin B_t deficiency. In the other 3, it is suggested that a relative deficiency of vitamin B_t may have existed. In 51 additional cases, without clinical evidence of vitamin B_t deficiency, the blood pyruvate was normal in every instance.

11418 P

A New Type of Vitamin K-Deficient Diets.

S. Ansbacher.

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Heretofore the vitamin K-deficient Diet E of Almquist and Stokstad,¹ Ration K-1 of Ausbacher,² Diet 508 of Dam and coworkers,³ or modifications thereof, have been used by the majority of investigators¹⁻⁷ in assays, in which chicks served as test animals Since putrified fish meal is an excellent source of one of the natural antihemorrhagic vitamins, obstacles are frequently encountered in the employ of fish meal diets even under conditions tending to minimize bacterial action

Recently we found that the difficulties arising from K-vitanin synthesis are not experienced when diets are used which contain neither fish meal nor yeast, and in which vitamin K had been destroyed by prolonged heat treatment. We are now making vitamin K assays with Ration K-7 outlined in the accompanying table

¹ Almquist, H. J., and Stokstid, E. L. R. J. Nutrition, 1936, 12, 329

² Ausbicher, S., J. Nutrition 1939, 17, 303

Dam, H., Glavind, J., and Kriter, P., Helt Chim Acta., 1940, 23 224

⁴ D mu, P. P., PROC SOC EXP BIOL AND MED 1939, 42, 663

⁵ MacFie, J. M., Bicharach, A. L., and Chance, M. R. A., Brit. Med. J., Dec. 23, 1939, 1220

of Theyer, S. A., McKee, R. W., Brukley, S. B., MacCorquodale, D. W., and

Doisy, E. A., PROC Soc Exp Biot and Med., 1939, 41, 194

⁷ Tidrick, R. T., Joyce, F. T., and Smith, H. P., PROC. Soc. Exp. Biol. and Med. 1939, 42, 853

RATION K-7.	%
Heated grain mixture* { wheat middlings	83
(yellow corn	12
Salt mixture ²	2
Calcium carbonate	1
Cod liver oil (medicinal)	2

*A mixture containing 25 parts of wheat middlings and 58 parts of yellow corn heated to 120°C for one week.

†To 1.2 kg of G.B.I. Vitamin-free Casein or of casein purified by washing with sodium chloride according to the method of either Supplee et al.11 or Edgar and collaborators 12, an aqueous solution is added containing 20 mg of thiamin chloride, 20 mg of riboflavin, 20 mg of vitamin B₆ hydrochloride and 1 g of nicotinic acid. The product is thoroughly mixed and then dried at a temperature not exceeding 70°C.

It is similar to the Ansbacher *et al.*⁸ modification of the 240-H ration of Kline *et al.*⁹ the outstanding difference being the heat treatment of the grain mixture which is prolonged from 36 to about 168 hours.

When baby chicks are fed the heat-treated diet, they grow only very slightly, have an increased blood clotting time and show the typical K-avitaminosis symptoms within from 4 to 8 days, and die within approximately 2 weeks. If it is intended to make vitamin K assays with chicks weighing about 70 g, it is necessary to start baby chicks on a vitamin K-low diet, e.g., Ration K-2,² and to feed Ration K-7 or a similar heat-treated diet after the birds attained the desired weight. Vitamin K-deficiency will occur after the fourth day on the K-7 diet, even when the chicks have access to feces, are housed in dirty cages and receive water contaminated with the diet.

Experiments with chicks have shown that Ration K-7 is not only deficient in vitamin K, but also in pantothenic acid and some other as yet unknown factor or group of factors. Therefore, it may be supplemented with a suitable vitamin B complex concentrate, such as c.g. an aqueous extract of rice bran similar to that used by Lepkovsky¹⁰ and known to be rich in pantothenic acid.

Preliminary results brought out the fact that Ration K-7 may be used as the basal diet for rats in studies of the anti-achromotrichia factor. When rats were fed this diet from weaning age on, graying of the hair occurred within 60 to 70 days. The absence of the anti-

Ansbacher, S., Supplee, G. C., and Bender, R. C., J. Nutrition. 1936, 11, 529.
 Kline, O. L., Keenan, J. A., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem., 932-33, 99, 295

¹⁰ Lepkovsky, S., Science, 1938, 87, 169; J. Biol. Chem., 1938, 124, 125.

¹¹ Supplee, G. C., Flanigan, G. E., Hanford, Z. M., and Ansbacher, S., J. Biol. Chem., 1936, 113, 787.

¹² Edgar, C. E., El Sadr. M. M., and Macrae, T. F., Biochem. J., 1938, 32, 2200.

achromotrichia factor could not be demonstrated, when the same ration without added crystalline vitamin B_0 was used.

It is to be expected that the new vitamin K-deficient diet will be found a useful tool in studies of some of the biological problems involved in vitamin K metabolism, since the diet apparently does not permit a bacterial K-vitamin synthesis.

11419

A Method for Staining of Carious Lesions in Teeth.*

G. Gomori. (Introduced by R. G. Bloch.)

From the Department of Medicine, University of Chicago.

In the study of experimental rat caries large numbers of animals must be employed to obtain significant statistical results. As a result the methods for examination of the molar teeth become very important. Some investigators have employed the very tedious method of preparing stained thin-sections by the ordinary technics. Others have attempted to reduce the time and expense by resorting to either gross inspection of the carious teeth or to rapid grinding and examination of ground sections. At the suggestion of Dr. B. F. Miller of the University of Chicago the author has developed a rapid, simple and precise method for the staining of carious areas in rat molars (and also in human teeth). Previously,1 the author had developed a method for the demonstration of insoluble calcium salts in the tissues. It was found that this method cannot be applied to the study of teeth because the silver solution used in the technic will not penetrate the dense dental tissues. Dentin will get a very superficial black coating but enamel is entirely inistained. However, the surprising observation was made that carious areas stained deep black. This can be explained by the greater permeability to the silver solution of the rarefied carious tissue. That the action of acid actually increases the permeability of enamel and dentin was proved by the following experiment: into healthy, extracted human teeth symmetrical holes were drilled, two into each. One of these

^{*} This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Gomeri, G., Am. J. Path., 1933, 9, 253.



Ground Surfaces of Human Teeth.

A. Proximal caries.

B. Secondary caries under a filling. C. Two holes drilled into a tooth. The left hole, treated with distilled water; the right hole, treated with lactic acid.

holes was packed with cotton soaked in distilled water, the other with cotton soaked in 5 to 10% lactic acid. The holes were sealed with paraffin. The cotton plugs were changed twice daily. After 3 days the plugs were removed, the teeth washed in distilled water and stained according to the technic mentioned. The holes that were treated with distilled water only did not show any staining by silver, whereas the holes packed with lactic acid were surrounded by a black area up to 2 mm in width (Fig. 1).

The silver impregnation method is very suitable for the examination of large numbers of rat jaws for carious lesions. The stained jaws may be directly examined under the surface microscope, or decalcified, embedded in celloidin and made into microscopic sections.

The method is as follows: 1. Fix in neutralized 80 to 95% alcohol or in neutral formalin. 2. Wash tissues in repeated changes of distilled water. 3. Impregnate with a 0.25 to 0.5% solution of silver nitrate for 12 to 24 hours. 4. Wash in many changes of distilled water for at least 24 hours. 5. Reduce in a 5% solution of sodium hypophosphite for 24 hours. 6. Wash under the tap for several hours. 7. Fix in a 2% solution of sodium thiosulfate (hypo) for 12 hours. 8. Wash under the tap for several hours.

The stained jaws may be either dehydrated in alcohol, cleared in cedar oil and examined directly under the dissecting microscope,;

[†] This variant of the technic has been used by Dr. B. F. Miller (Proc. Soc. Exp. Biol. and Med., 1938, 39, 389).

achromotrichia factor could not be demonstrated, when the same ration without added crystalline vitamin B_0 was used.

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^{*} This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

¹ Gomori, G., Am. J. Path., 1933, 9, 253.

dentin at the tips of the cusps however, often shows a more or less superficial staining. Examination of the sections for lesions is very easy because the carious areas take such an intense, conspicuous black stain.

For decalcification one should employ a 5 to 10% solution of sulfosalicylic acid instead of mineral acids which attack the silver deposits.

The author wishes to express his appreciation to Dr. J. R. Blayney and to Dr. B. F. Miller of the Walter G. Zoller Memorial Dental Clinic for their valuable suggestions.

11420 P

Susceptibility of Field Mice and Meadow Mice to St. Louis Encephalitis.

J. E. Greutter, J. D. Fulton, R. O. Muether, E. V. Hanss and G. O. Broun.

From the Department of Internal Medicine, and the Department of Biology, St. Louis University School of Medicine.

In the vicinity of St. Louis the 3 most common species of wild mice are the field mouse, Reithrodontomys megalotis, the house mouse Mus musculus and the meadow mouse, Microtus ochrogaster. As long ago as the epidemic of 1933 efforts were made to trap mice in the homes of encephalitic patients. Several field mice were captured but no representative of either of the other species were obtained in such homes. Beginning in 1934 we tested the susceptibility of field mice to the virus of St. Louis encephalitis and found that they can be infected both by intracerebral and intranasal inoculation. Harford, Sulkin and Bronfenbrenner¹ have reported that the house mouse, Mus musculus, is also susceptible to this infection.

More recently we have captured a large number of field mice and also have been able to capture a number of meadow mice. Tests for the susceptibility of these strains of mice to the encephalitic virus have been carried out using simultaneous tests on white Swiss mice for comparison.

¹ Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J., PROC. Soc. Exp. Biol. AND Med., 1939, 41, 331.

or they may be decalcified and embedded in celloidin. Six or more jaws may be included in a single celloidin block. The sections can be counterstained with any stain desired. Sections of several to many dozens of rat jaws depending on the number of sections required, can be cut, stained and mounted in a few hours.

In the finished sections the carious areas are stained deep black (Figs. 2 and 3). A superficial layer of bone and dentin, up to about 20 micra in thickness, will usually be stained black or dark brown, and, in addition, some superficial osteoblasts with their processes. Calcium-containing debris in the fissures will show up as a black, finely granular mass. Healthy enamel is unstained, the exposed



Fig. 2. Large destructive lesion in a rat molar. \times 17.5.



Fig. 3. Small, deep lesion in a rat molar, starting at the side of a cusp. imes 35.

11421 P

Observations Concerning Culex pipiens as a Possible Carrier of St. Louis Encephalitis.

J. D. Fulton, J. E. Greutter, R. O. Muether, E. B. Hanss and G. O. Broun.

From the Department of Internal Medicine, St. Louis University School of Medicine.

It has been shown by Casey and Broun that St Louis encephalitis cases appear to occur with higher incidence in those areas of the city and county which are adjacent to small streams and open ditches.¹ This suggests the possibility of a water breeding insect as a possible transmitting agent. Transmission of the equine types of encephalomyelitis by mosquitoes from animal to animal has been demonstrated by Kelser², Simmons, Reynolds and Cornell,³ and Merrill, Lacaillade and Ten Broeck.⁴

Webster, Clow and Bauer³ demonstrated the St. Louis encepha litis virus could be taken into the body of the *Anopheles quadrimaculatus* mosquito and retained for the duration of their lives. The virus containing mosquitoes, however, did not infect mice or monkeys by biting. Attempts were made subsequent to the 1933 epidemic to transmit encephalitis from human being to human being by the bite of the various species of mosquito without success.⁶

Since Culex pipiens is the most common type of mosquito in the St. Louis area, we have studied the ability of this mosquito to become infected with the virus of St. Louis encephalitis.

Mice infected with St. Louis encephalitis by intraperitoneal injection of heavy doses of virus have been shown by Webster and his co-workers to have a considerable concentration of the virus in the circulating blood for a period of five hours after the injection. In our experiments 1 cc of a 1/10 dilution of virus containing brain was injected intraperitoneally. The mouse was then placed in a specially built biting cage where he was exposed to a number of mosquitoes for a period of 5 hours after dark in a quiet room.

¹ Casey, A. E., and Broun, G. O., Science, 1938, 88, 450.

² Kelser, R. A., Jr., A. V. M. A., 1933, 35, 767.

³ Simmons, J. S., Reynolds, F. H., and Cornell, V. H., Am. J. Trop. Med., 1936, 16, 289.

⁴ Merrill, M. H., Lacaillade, C. W., and Ten Broeck, Carl, Science, 1934, 80, 251.

⁵ Webster, L. T., Clow, A. D., and Bauer, J. H., J. Exp. Med., 1935, 61, 479.

⁶ Report on the St. Louis Outbreak of Encephalitis, Public Health Bulletin, No. 214, United States Public Health Service.

The effect of the intracerebral injection of 0.030 cc of St. Louis encephalitic virus in dilutions of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} were tried in each species. In the case of the field mice some survivals were noted even in dilutions of 10^{-2} and the majority of the animals survived in dilutions of 10^{-4} , 10^{-5} , and 10^{-6} . In the case of white Swiss mice controls, there were no survivals in less than 10^{-6} . In meadow mice, no survivals were found in dilutions lower than 10^{-4} .

In one experiment using a number of resistant field mice, it was possible to show that animals inoculated intracerebrally with the virus showed no evidence of illness 10 days after the injection, yet were carriers of the virus in their brain tissue since the injection of this brain tissue into Swiss mice regularly resulted in the production of encephalitis. The brain emulsion of the first field mouse was then transferred to a second field mouse. This animal also remained well. Again after a period of 10 days, the brain of the second animal was shown to contain the virus when transferred to white mice. In like manner, the virus was carried through 4 successive transfers in field mice, the brain emulsion producing fatal encephalitis in white mice in each instance.

While we have captured a number of field mice in the homes of encephalitis patients, in no instance have we been able to detect the presence of the virus in the brain, naso-pharynx, spleen or other tissues in any of these animals. We have also examined the tissues of numerous field mice captured at random in various parts of St. Louis and St. Louis County, and in no instance have we been able to show that these animals are carriers of the virus. Field mice and meadow mice while a potential reservoir of encephalitis virus have not been shown to harbor the infection spontaneously.

11422

Validity of Chemical Balance Studies in Eviscerated Animals, as Index of Carbohydrate Utilization.

SAMUEL SOSKIN, R. LEVINE AND M. TAUBENHAUS.

From the Department of Metabolism and Endocrinology,* Michael Reese Hospital, and the Department of Physiology, University of Chicago, Chicago, Ill.

We have recently employed the chemical balance method in the abdominally eviscerated animal, to study the utilization of carbohydrate by the peripheral tissues of dogs under the following conditions (a) normal and depancreatized;¹ (b) normals treated with insulin;^{2,3} (c) normals treated with an anterior pituitary extract;⁴ (d) hypophysectomized;^{4,5} (e) phlorhidzinized,⁶ The chemical balance was struck from the blood sugar, blood lactic acid and the muscle glycogen values at the beginning and end of the experiments; and from the amount of sugar administered during the experiment. The calculations from these data were based upon distribution ratios reported by others, namely, 1/6 of the body weight for sugar and lactic acid^{7,8} and 1/2 of the body weight for muscle glycogen.^{9,10}

There are 2 possible objections to the manner in which we calculated the utilization of carbohydrate by the muscles: (1) The possible presence of significant amounts of free sugar or of higher carbohydrate intermediates in the muscles, which would not be taken into account by glycogen estimations alone. (2) The possible presence of amounts of lactic acid in the muscles, which are not in equilibrium with the blood values. We have therefore repeated utilization experiments on eviscerated normal and depancreatized

^{*} Aided by the Max Pam Fund for Metabolic Research.

¹ Soskin, S., and Levine, R., Am. J. Physiol., 1937, 120, 761.

² Soskin, S., and Levine, R., Am. J. Physiol., 1938, 123, 192.

³ Soskin, S., and Levine, R., Am. J. Physiol., 1940, in press.

⁴ Soskin, S., Levine, R., and Lehmann, W., Am. J. Physiol., 1939, 127, 463.

⁵ Soskin, S., Levine, R., and Heller, R. E., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 6.

⁶ Soskin, S., Levine, R., and Lehmann, W., Proc. Soc. Exp. Biol. And Med., 1938, 39, 442.

⁷ Burn, J. H., and Dale, H. H., J. Physiol., 1924, 59, 164.

⁸ Lavietes, P. H., Bourdillon, J., and Klinghoffer, K., J. Clin. Invest., 1936, 15, 261.

⁹ Best, C. H., Dale, H. H., Hoet, J. P., and Marks, H. P., Proc. Royal Soc. London B, 1926, 100, 55.

¹⁰ Dye, J. A., and Chidsey, J. L., Am. J. Physiol., 1939, 126, P482.

At the end of this time the engorged mosquitoes were separated from the unengorged and placed in another cage. Small culture dishes of semi-stagmant water were placed on the floor of the cage to provide a place for the deposition of any eggs that might be laid and as a moisture source for the mosquitoes.

A number of engorged mosquitoes were selected at random and killed in ether fumes. Usually 3 mosquitoes were ground in a mortar and diluted with 10 cc of salt free broth, and then passed through a Berkefeld filter. Serial dilutions of 1:5, 1:10, 1:100 were made of the filtrates and each injected intracerebrally into 3 Swiss mice in 0.03 cc amounts. The injected animals were observed for 15 days after injection.

In 29 series of experiments in which the mosquitoes which previously had engorged on infected mice were macerated and injected into normal mice, some of the injected mice died in 10 instances. In only 3 cases, however, were we able to carry the virus in serial transfers and to prove conclusively by neutralization tests that the virus of St. Louis encephalitis had been the infecting agent.

In the successful experiments we have so far shown survival of the virus in the body of the mosquito no longer than 10 days. The concentration of the virus within the body of the mosquito has not been shown to be greater than 100 intracerebral lethal mouse doses. This is a very much smaller concentration than was shown by Webster to be present in the body of the Anopheles mosquito.⁵

Two series of experiments were conducted in which larvæ of about the first or second instar were placed in water containing a macerated infected brain suspension. The dilution of the brain suspension in water was 1:10. When these larvæ transformed into the adult form, the mosquitoes were macerated in broth, filtered and injected into normal mice. None of the mice showed any evidence of infection after such injections.

Nine series of experiments were conducted in which eggs laid by mosquitoes which had previously been allowed to feed on infected nuce were macerated in broth, filtered and injected into normal nuce.⁴ In all cases the mice remained normal and healthy.

In 15 experients normal mice were exposed in the biting cages to 50 to 100 mosquitoes which had previously been allowed to feed on an infected mouse and then allowed to stand until they became empty. They were then observed until they had been allowed to feed on the normal mice. The mice were allowed to remain in the cage for 12 hours and then removed. In all cases the mice were healthy and normal after 15 days.

TABLE I. Summary of Data for Alternative Calculations of Carbohydrate Utilization by Eviscerated Normal and Depancreatized Dogs Respectively.

			Dura	ıtion	Total	B	gus poo	ar	Blo lactic	Blood actic acid	Total CHO in musele	OHO Isele	Lacti in m	Lactic acid in musclo
Dog	No.	Wt, kg	H L	or exp. Hr Min	sugar injected, g	Initial mg%	l, Final, mg%	Initial, Final, Avg, mg% mg% mg%	Initial, mg%	nitial, Final, mg% mg%	Initial, Final, mg% mg%	Final, mg%	Initial, mg%	Initial, Final, mg% mg%
Norm.	1	11.4	4	0	5.46	84	122	92	32.4	129.0	449.9	290.3	37.4	72.6
1.5	C 3	9.7	*	0	6.97	236	277	262	47.2	125.0	461.7	264.0	12.9	56.3
11	m	9.4	က	0	9.50	474	675	574	32.4	61.0	581.5	399.5	25.0	34.5
Dep.	4	8.4	က	0	1,60	202	175	193	51.2	111.6	470.0	392.0	97.0	98.0
	FD.	12.5	က	0	14.90	337	646	206	132.8	160.0	431.6	387.3	154.8	102.2
1.5	9	7.4	¢1	40	1.59	390	392	403	77.0	204.0	443.0	207.0	54.2	148.6

258		(СН	01	Util	IZAT	101	ву (Сие	MICA
ated as 50% of body wto equals 900 ec. In calculation (B), the Extracedular fluid of muselc	Calculation (B)		2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	1.09 × 2.000 = 9063 mg	Out	0010 >> 00	, 208 = 802 ·	700	1010 / * * * *	100 = 2038 "
We il.4 kg. Exp. period 4 hr. Avy blood sugar throughout exp. way 92 mg%. Muscle mass calculated as 50% of body wto equals 5700 g. In calculation (A) the blood and extracellular fluid is taken as 16 of the body wt.7.3 equal to 1900 ec. In calculation (B), the blood and extracellular fluid is considered equal to 30% of body wt.13.14 Blood volume equals 7%. Extracellular fluid of muscle (30—7)/2 equals 11.5%. Blood plus non-muscle extracellular fluid equals 18.5%, or 2109 ec.		Initial total CHO of musele 419.9 mg%	Difference 159.0 "	Tatal CHO decrease	Initial blood angar 84.0 "	Diff. 38.0 "	Increase in extracellular sugar	Initial blood lactic acid 32.4 "	Diff. 96.6 "	Increase in extracellular lactic acid
Wt 11.4 kg. Exp. period 4 l 5700 g. In calculation (A) the blood and extracellular fluid is (30 7)/2 equals 11.5%. Bloo	Calculation (A)	er karameter e versamenter er karameter er karameter er karameter er karameter er karameter er karameter er ka		109 × 5,000 100 == 9063 mg		38 × 1900	200 mg = 729 mg		96.6×1900	100 = 1835 mg

AL BALANCE . . • . 5155 9672 - := 2006 48.16 = 14518(9063 + 5455) = (802 + 2038 + 2006) = (14518 - 4846) 35.2×5700 100 3 = . . Dextrose injected during experiment Total sugar disposed of Total retained 11gar 37.4 73.6 Inerense in muscle lactic acid Diff. Sugar utilized Initial unusele luctic acid Eig. Fint

:

212

11.4×4.11

9672

Sugar utilized per kg original body wt per hr

,

 $\begin{pmatrix} 9063 + 5455 \end{pmatrix}$ $722 + 1835 \end{pmatrix}$ (14518 - 2557)

= 262 mg

 11.4×4 11961

5455 r = 14518 = 2557= 11961 2557

T TOTAL STREET	July Miller For	101 41114											-	
									Blo	od	Total CIIO	Oito	Lacti	c acid
			Dur	ation	Total	131	Bus poo	นา	Inctic	neid	וווו ווו	selo	in m	nselo
		Wt.	i (01 exp.	injected,	Initial	Initial, Pinal, Avg,	Avg,	Initial, Plual.	Flual,	Initial, Pinal,	Final,	Initin!	Initial, Final,
Dog	No.	, R	III	Min	ec.	% Hu	mg%	mg%	mg%	mg%	% Bm	% Ym	,%Su	mg%
Norm.	1	11.4	-	0	5.46	84	133	95	39.4	129.0	449.0	290.3	37.4	72.6
•	21	9.7	*	0	6.07	536	277	300	47.2	125.0	401.7	204.0	12.0	56.3
:	**	9.4		0	9.50	₹2₹	675	574	33.4	61.0	581.5	300.5	25,0	34.5
Dep.	÷	8.4	ç	0	1.60	202	17.5	193	51.5	111.6	.170.0	392,0	97.0	080
<u>.</u>	r	12,5	cc	=	14.90	337	646	506	132.8	100.0	431.6	387.3	154,8	102.3
=	9	7.4	¢1	Ç	1.59	390	393	403	77.0	204.0	443.0	207.0	54.3	148.0
-			-	-				-			***************************************			

dogs respectively, in which we have determined the total carbohydrate content of the muscle instead of muscle glycogen, and the lactic acid content of the muscle as well as blood lactic acid. Our results by these methods are presented, and are compared with our previous results.

Methods and Results. The details regarding the technic of these experiments and the chemical methods employed were described in a previous publication.\(^1\) The total carbohydrate estimations in the present work were made by the method of Tsai, as modified by Benoy and Elliott.\(^{11}\) The blood and muscle lactic acids were determined by a slight modification of the method of Miller and Muntz.\(^{12}\)

The data are summarized in Table I. Calculations of utilization from these data were made in 2 ways: (A) depending on the distribution ratios used in our previous work, (B) treating blood and muscle separately. These 2 types of calculation are exemplified in parallel, for animal No. 1.

It may be seen that the results of the above calculations, although they show some difference, are of the same order of magnitude. Fig. 1 graphically represents the results of both calculations for all our present animals, and offers a comparison with our previously published results and calculations. The present methods of calculating introduce only a slight and consistent difference in the data as a whole. It is understood, of course, that none of the above methods can be held to yield absolute utilization values. But it is equally evident that any of them give good comparative results.

Discussion and Summary. It is clear that neither of the possible objections to our previous method of calculating carbohydrate utilization is sufficiently valid to materially affect the end results. That is, the amounts of free sugar, higher carbohydrate intermediates, or lactic acid present in the muscles are not such as to invalidate calculations based on blood sugar and lactic acid values, and distribution ratios. This agrees with the earlier, basic work of Best, Dale, Hoet and Marks, who demonstrated that the sugar which disappeared from the blood of eviscerated spinal cats was equal to the sum of the glycogen deposited in the muscles and the glucose equivalent of the oxygen consumed.

The close correspondence of utilization rates, as determined by

¹¹ Benoy, M. P., and Elliott, K. A. C., Biochem. J., 1937, 31, 1268.

¹² Miller, B. F., and Muntz, J. A., J. Biol. Chem., 1938, 126, 413.

¹³ Chidsey, J. L., and Dye, J. A., Am. J. Physiol., 1939, 126, P461.

¹⁴ Peters, J. P., Body Water, Springfield, C. C. Thomas, 1935, p. 138.

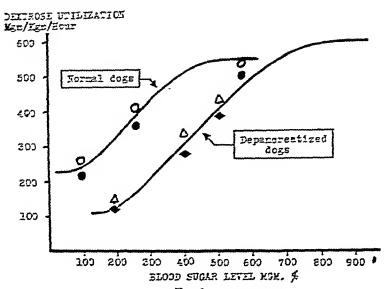


Fig. 1.

Comparison of Carbohydrate Utilization by Normal and Depancreatized Dogs by

Different Calculations.

The plotted points represent the results of the present work and calculations, and are not the points through which the smooth curres are drawn. The curres are included for comparison, and are derived from previous works on the rates of sugar utilization by the entra-hepatic tissues of normal and departmentized dogs, respectively.

- Normal dogs. calculation (A).
- Normal dogs, calculation (B).
- △ D-panereasized dogs, calculation (A).
- Departmentized dogs, calculation (B).

different methods and by different calculations, strongly supports the validity of the chemical balance method as a means of determining carbohydrate utilization in liverless animals. dogs respectively, in which we have determined the total carbohydrate content of the muscle instead of muscle glycogen, and the lactic acid content of the muscle as well as blood lactic acid. Our results by these methods are presented, and are compared with our previous results.

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before injection with 0.1 N NaOH. All injections were given intramuscularly.

A modified Tillman's procedure was used for the estimation of the ascorbic-acid content of the serum. The method follows: 4 to 5 cc of blood were drawn by cardiac puncture and allowed to stand for 15 minutes and centrifugalized. Two cc of the serum was then added to 4 cc of freshly prepared 20% trichloracetic acid. The mixture was allowed to stand for 5 minutes and again centrifugalized. Three cc of the clear supernatant liquid was then titrated against 2,6-dichloro-phenolindophenol (Hoffbann-LaRoches tablets). Each cc of the dye solution is equivalent to 0.02 mg of ascorbic acid. All titrations were done in duplicate and triple distilled water was always employed.

TABLE I.

Daily Dose of Ascorbic Acid and Blood-serum Ascorbic Acid in the Guinea Pig.

376	70 - 11 - 1	Blood-ser	um ascorbic a	cid in mg%
No. of leterminations	Daily dose, mg	min.	max,	med.
40	0	0	.05	.02
220	0.5	0	.21	.09
218	1.0	.05	.36	.13
47	2.0	.12	.52	.33
117	5.0	.39	.74	.57
143	10.0	.83	1.44	1.03
85	20.0	.87	1.73	1.08

Results. The table gives a composite protocol of the results of 870 blood-serum ascorbic-acid values.

It is seen that the maximal concentration of vitamin C in the serum of the guinea pig only occurs when a daily dose of from 10 to 20 mg is given. Since it has been shown that serum-complement reaches its maximal activity at a level of about 1 mg of ascorbic acid per 100 cc, it is suggested that an amount of 10 mg per day be given to maintain the normal activity of the blood-serum proteins since a full complementary activity is an expression of the normal behavior of these proteins. Cohen⁶ found that 5 mg of ascorbic acid per day was needed in a tooth-protective ration.

Summary. Analysis of a series of 870 blood ascorbic-acid values reveals that a daily intake of from 10-20 mg of ascorbic acid is needed to secure a 1 mg per 100 cc concentration in the blood serum of the guinea pig. Under these conditions a normal complement-value is obtained.

⁶ Cohen, M. B., J. Allergy, 1938, 10, 15.

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Vitamin C Requirement of the Guinea Pig.*

E. E. ECKER AND L. PILLEMER.

From the Institute of Pathology and the University Hospitals of Western Reserve University, Cleveland, Ohio.

The assumed daily requirement of ascorbic acid for the guinea pig has gradually been increased from 0.5 mg to 1.5 mg (Szent-Györgyi.¹) In fact, Zilva² showed that even on a dosage of 1.5 mg per day saturation of the tissues did not occur, and that about 20 times this amount was necessary to maintain saturation of the tissues. However, since Zilva's animals apparently thrived on 1.5 mg per day, this author assumed that this amount sufficed to assure undisturbed growth and health.

Szent-Györgyi,¹ however, believes that the true physiologic daily dose of the vitamin is that amount which the animal consumes in its native environment. He calculated that in his natural habitat the guinea pig ingests about 30 mg of ascorbic acid daily, and that this amount compares favorably with the amount needed to maintain a maximal saturation of its tissues. During the course of the experiments on the relationship of vitamin C to complementary activity,³-⁵ 870 blood-serum ascorbic-acid determinations were made. The guinea pigs were given varying amounts of ascorbic acid.

This report deals with the relationship of the blood-serum ascorbic-acid level to the daily intake of varying amounts of the vitamin.

Methods. All the animals were maintained on a stock diet of Purina mixed rabbit chow. This mixture is supposed to contain all the essential elements needed for full growth of the animals with the exception of vitamin C. In fact, guinea pigs develop scurvy when maintained on this diet for periods longer than 21 days.

Crystalline ascorbic acid was administered either as a commercial sodium salt known as "Cevalin" or as ascorbic acid neutralized

^{*} Aided by a grant from the Commonwealth Fund.

¹ Szent-Györgyi, A., Deutsch. med. Wehnschr., 1937, 63, 1789.

² Zilva, S. S., Biochem. J., 1936, 30, 1419.

³ Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., J. Immunol., 1938, 34, 19.

⁴ Pillemer, L., Seifter, J., Kuehn, A. O., and Ecker, E. E., to be published.

⁵ MacDonald, F., and Johnson, H., to be published.

serum was then suspended in a large stoppered test tube containing 50 cc of cold 1.0 N NaCl solution. The sac was suspended in the saline at such a height that the level of the saline was about a centimeter above the level of the serum within the sac. A control sample of the same guinea-pig serum was measured into a similar cellophane sac and sealed in the same manner as above. This was suspended in an empty, large, stoppered test tube.

The large test tubes containing the sacs were placed in a cold room at 4.5°C. At the end of 24 hours, the sacs were opened and 0.05 cc of serum was removed from each sac. These samples were diluted 1:30 with 0.9% NaCl solution and the complement-activity of each sample was determined by the method of initial hemolysis of Ecker et al.2 After removal of the samples for titration, the cellophane sacs were again sealed. Each serum undergoing dialysis was suspended in another 50 cc portion of cold 1.0 N NaCl solution. Dialysis was allowed to proceed at 4.5°C for another 24-hour period at the end of which time the above procedure was repeated. was continued throughout the course of the experiment. volume of each dialyzed serum and of the control serum was measured at intervals during the experiment. It was found that in no case did the volume increase by as much as 10% during the entire experiment. There was no change in the volume of the control.

The table shows the complement values obtained for three dialyses of 2 different serums, and also the titration values for the controls.

Conclusions. It is seen from the above table that guinea-pig serum slowly loses some of its complementary activity during dialysis against 1.0 N NaCl solution at a low temperature over a period of 2 weeks. This loss in activity is in no way comparable, however, with that reported by Chow and Zia who stated that in a

Complement-activity of Serum During Dialysis in the Cold Against 1.0 N Sodiumchloride Solution.

Period of dialysis in hr	0	24	48	72	96	144	168	192	216	240	264
Dialyzed serum A Control A Dialyzed serum B ₁ Dialyzed serum B ₂ Control B	.03 .03 .02 .02 .02		.03— .03+ .03 .03		.04+		.03 .03	.08 .07+	.04 .04 .04	.08	.03 .03 .03

² Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., J. Immunol., 1938, 34, 19.

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Dialysis of Complement Against 1.0 Normal Sodium Chloride.*

Chase Breese Jones and E. E. Ecker.

From the Institute of Pathology and the University Hospitals of Western Reserve University, Cleveland, Ohio.

Chow and Zia¹ reported that when guinea-pig serum is dialyzed against normal NaCl solution through a cellophane membrane at about 5°C its complementary activity rapidly decreases. They also stated that they were able to restore practically all of the original activity of the serum by combining the dialysate with the non-dialyzable fraction, and concluded that the loss of activity was due to the removal of a dialyzable component of the complement. Since these authors were unable to reactivate dialyzed serum by the addition of yeast-inactivated complement, or ammonia-inactivated complement, or by complement inactivated hy oxidation with iodine, but were able to reactivate with dialysates of serums which had previously heen inactivated hy any of the above methods, they state that "these results, therefore, indicate that the dialyzable component of complement is different from the third or fourth or the oxidizable components of complement."

Because of the introduction of a new factor or factors in the constitution of complement it was thought of interest to study the reactivation of complement which had been inactivated by dialysis. However, upon repetition of the experiments of Chow and Zia, it was not possible to confirm their results in respect to the inactivation of complement by dialysis under the conditions reported by them.

It was observed that serum dialyzed in the cold (4.5°C) against 1.0 N NaCl, even over long periods of time, loses its hemolytic activity no more rapidly than serum which is stored without dialysis under the same conditions.

Experimental. Five cc of fresh guinea pig serum were measured into a 5%-inch cellophane tube (No. 341), the bottom of which had been folded and tied off with silk thread in such a way as to prevent leaking. The top of the tube was sealed with a rubber stopper and tightened with a rubber band. The cellophane sac containing the

^{*} Aided by a grant from the Commonwealth Fund.

¹ Chow, B. F., and Zia, S. H., PROC. Soc. Exp. Biol. And Med., 1938, 88, 695.

to the amount consumed by the rat when the butter fat is incorporated in the diet at a level of 9%. Butter fat is thus placed in the peculiar position of being an anti-dermatitis agent when fed as a supplement, and of failing to exhibit this property when mixed with the diet. This anomalous rôle of butter fat was clarified by the following experiments. These indicate that the mixing of butter fat in the diet may result in a destruction of the anti-dermatitis potency of the former when the mixed diet is allowed to stand in contact with the air for periods of time usually met with in the laboratory production of rat dermatitis.

Experimental. The diet used had the following composition. Glucose 68, casein (alcohol extracted) 18, salts 4, cod liver oil 1, butter fat 9. In addition each rat received the following daily supplements: 5 µg calciferol and 10 µg beta carotene in 1 drop of the liquid fraction of hydrogenated coconut oil, plus 20µg riboflavin and 10 µg thiamin chloride hydrochloride in 1 drop of N/50 acetic acid. The diet, apart from vitamin supplements, was fed in two forms, "fresh" and "rancid." The "fresh" diet was made up weekly, previously prepared but unused quantities being discarded, and was stored in a refrigerator between feedings. The "rancid" diet was made up one month before use and was stored for that period, open to the air, in a warm room When used the "rancid" diet had the characteristic odor of rancidity (Peroxide No. of ether extracted fat was 131). Diets were fed daily and uneaten portions discarded.

The rats used were 35-40 g weanlings specially prepared³ on a diet low in anti-dermatitis factors. When 3 rats were fed the "rancid" diet they developed a florid dermatitis in 6 weeks. Three rats fed the "fresh" diet failed to develop any symptoms of dermatitis even after 15 weeks. When the rats which had developed dermatitis on the "rancid" diet were changed to the "fresh" diet, the dermatitis was cured in three weeks. When the experiment was repeated with the same number of animals using the paired feeding method the results were the same. The differences thus observed could not be attributed to differences in consumption of the diets.

Summary. The anti-dermatitis action of fresh butter fat has been confirmed.

Destruction of the anti-dermatitis potency of butter fat has been demonstrated in a diet in which the butter fat was allowed to become rancid.

³ Quackenbush, F. W., Platz, B. R., and Steenbock, H., J. Nutr. 1939, 17, 115.

typical experiment a sample of serum lost nearly 90% of its original activity upon dialysis for 4 days under the same experimental conditions, while a control sample lost only 25% of its activity during storage for the same period. It will be noted from the results of the present experiments that both the total loss and the rate of loss of complement activity in the dialyzed serum is in each case substantially identical with that of the undialyzed control serum. It is upon this evidence that the conclusion is drawn that none of the components of complement are removed from the serum by dialysis against 1.0 N NaCl. The loss of complementary activity obtained by allowing serum to remain in the cold for long periods of time may be explained upon the basis of oxidation alone, or other factors, since neither complement nor any of its parts is dialyzable through a cellophane membrane against 1.0 N NaCl solution in the cold. In additional experiments, little loss of activity was obtained in dialyzing against cold 1.0 N NaCl solution complement which had been purified by a fractional precipitation method which will be reported in a subsequent paper. Dialysis in this case has been allowed to proceed for periods as long as 10 days.

Summary. Dialysis against 1.0 N NaCl at 4.5°C does not remove a dialyzable component from the guinea-pig complement. The gradual loss of activity noted may be accounted for on the basis of changes within the protein-molecule.

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Butter Fat in Dermatitis-Producing Diets.

HOWARD A. SCHNEIDER. (Introduced by D. D. Van Slyke.)

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison, Wis.*

Butter fat has been revealed both as a curative agent¹ for rat dermatitis and also as a component (9%) of a dermatitis-producing diet.² The curative property was demonstrated by a daily supplement of 500 mg of fresh butter fat, a quantity approximately equal

^{*} Present address: Hospital of the Rockefeller Institute for Medical Research, New York.

¹ Schneider, H. A., Ascham, J. K., Platz, B. R., and Steenbock, H., J. Nutr.,

² György, P., Biochem. J., 1935, 29, 741.

pH determinations done with the glass electrode show that, in the amounts used, the change in pH caused by addition of the sodium salts is small.

Cultures were incubated at 37.5°C.

Readings were made at magnification of 60X with a standardized ocular micrometer using a mechanical stage (114 units = 1 mm). The average colony diameter was used as the index of bacteriostasis. Colony counts per culture are also given in the table.

The effect of 3 concentrations of each drug was studied for each strain. With one exception readings were made at or near the 24-hour period. The results are given in Table I.

It will be noted that even on the weight basis sulfathiazol compares favorably with sulfanilamide in inhibiting the growth of both strains studied. Sulfamethylthiazol is somewhat less effective.

When used in sufficient concentration to cause marked bacteriostasis, both drugs inhibit the development of the normal diffuse periphery usually seen around colonies growing in this medium. The periphery may be completely inhibited or it may be composed of a loose net of long, heavy chains of streptococci which are never seen in the controls. This influence on the periphery has been described previously for sulfanilamide in this medium by King, Henschel and Green.⁴ Others have noted the formation of such chains in fluid media.

Sulfamethylthiazol is less effective than sulfathiazol in inhibiting the development of the normal diffuse periphery.

Strains which we have studied previously have usually not shown a significant decrease in the number of colonies when growing in tissue culture clots containing sulfanilamide. In the highest con-

Action of Sulfanilamide (S), Sulfathiazol (ST), and Sulfamethylthiazol (SMT) on Two Strains of Beta Streptococci; 22-24 Hours Except as Noted.

Drug cone.	mg% 50		10		1	
	No. Cols.	Diam.	No. Cols.	Diam.	No. Cols.	Diam
		Str	ain C203.			*******
Control	44.2	60.8	40.7	38.7	23.1	45.1
S	5.2	5.6	20.2	20.9	18.6	42.3
rs	2.5	5.6	22.2	15.6	23.5	49.2
SMT	16.2	11.6	31.0	24.8	20.2	49.0
		Stra	in No. 40.			
Control	24.7	77.7	84.2	73.4*	53.6	85.4
S	14.5	9.4	85.2	22.8	52.3	57.2
ST	6.2	5.8	89.3	19.7	53.6	72.0
SMT	8.0	8.4	92.3	26.6	50,6	83.4

^{* 10} mg% conc. read at 48 hours.

Baeteriostatic Effect of Sulfathiazol and Sulfamethylthiazol for Beta Hemolytic Streptococci in Tissue Culture Clots.*

JOSEPH T. KING AND AUSTIN F. HENSCHEL.

From the Department of Physiology, University of Minnesota, Minneapolis.

The bacteriostatic effect of 2(para-amino-benzene-sulfonamido) thiazol (sulfathiazol) and 2(para-amino-benzene-sulfonamido) 4-methylthiazol (sulfamethylthiazol) has been compared with that of sulfamilamide. Two strains of beta streptococci have been used, C 203 and No. 40. Strain C 203 has been used in many studies reported in the literature. Strain No. 40 is a Lancefield group C of human origin which has been studied extensively in this laboratory.

The Maximow culture technic was used. Details have been described previously.¹⁻¹ A culture consists of one drop of heparinized rabbit plasma and 3 drops of rabbit serum extract of 7-day chick embryos.

The extract was inoculated with a culture of streptococci grown in the serum extract described above to which 5% of rabbit erythrocytes were added. Cultures are grown only until hemolysis occurs. For strain No. 40 this requires 3-5 hours and somewhat longer for C 203. Dilution of the bacterial culture was made rapidly through Tyrode into the extract to make a final dilution of 10⁻⁶. The inoculated extract was then divided into 4 equal portions and equal volumes of the designated drug added to each of the experimental tubes and the same volume of Tyrode to the control.

A 400 mg % stock solution of each drug was sterilized by Berkefeld filtration through a filter used only for that drug. Solutions were kept in the ice box.

Due to the limited solubility of the thiazol derivatives these were used as the sodium salts.

^{*} Aided by grants from the Medical Research Fund, Graduate School, the University of Minnesota, and the Department of Medical Research, the Winthrop Chemical Co., Inc. All drugs supplied by the Winthrop Chemical Co., Inc. Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, official project No. 65-1-71-140, sub-project No. 237, and by the National Youth Administration.

¹ King, J. T., Arch. f. Exp. Zellforsch., 1930, 9, 341.

² King, J. T., Arch. f. Exp. Zellforsch., 1931, 10, 467. ³ King, J. T., Arch. f. Exp. Zellforsch., 1937, 20, 208.

⁴ King, J. T., Henschel, A. F., and Green, B. S., J. Am. Med. Assn., 1939, 113, 1704.

pH determinations done with the glass electrode show that, in the amounts used, the change in pH caused by addition of the sodium salts is small.

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SMT	16.2	11.6	31.0	24.8	20.2	49.0
		Stra	in No. 40.			
Control	24.7	77.7	84.2	73.4*	53.6	85.4
S	14.5	9.4	85.2	22.8	52.3	57.2
ST	6.2	5.8	89.3	19.7	53.6	72.0
SMT	8.0	8.4	92.3	26.6	50,6	83.4

^{* 10} mg% conc. read at 48 hours.

centration of the drugs used in this study, strain No. 40 did show a decrease in colony count at 24 hours. By 48 hours, however, the counts had increased to 22.5 in cultures containing sulfanilamide, 20.5 in sulfathiazol and 21.7 in sulfamethylthiazol (controls 24.7).

Strain C 203, which we are using for the first time, shows a decrease in colony count at 24 hours not only in the 50 mg% concentration but also in 10 mg%. Furthermore, the count in the 10 mg% concentration had increased to only 32.7 in sulfanilamide, 31.0 in sulfathiazol and 38.7 in sulfamethylthiazol at 72 hours (control 40.7). In the 50 mg concentration there was no significant increase in average number of colonies per culture at 5 days. Some of the few colonies which were present continued to grow very slowly, however. This strain is definitely more susceptible in this respect than strains which we have studied previously.

Due to differences in method of assay and drug-organism combinations, it is difficult at this time to compare the results of *in vitro* tests. It is now generally realized that the medium used and the size of the inoculum markedly influence the results obtained.

Lawrence⁵ found sulfathiazol and sulfamethylthiazol superior to sulfanilamide in their bacteriostatic effect against group A hemolytic streptococci. Long and Bliss⁶ found sulfathiazol to be as effective as sulfanilamide against a number of organisms including group A streptococci.

Summary. Under the experimental conditions employed sulfathiazol is as effective a bacteriostatic agent against the strains of beta hemolytic streptococci tested as is sulfanilamide. Sulfamethylthiazol is somewhat less effective.

⁵ Lawrence, C. A., Proc. Soc. Exp. Biol. and Med., 1940, 43, 92.

⁶ Long, P. H., and Bliss, E. A., PROC. Soc. Exp. Biol. And Med., 1940, 43, 324.

11427

Growth of Bacteria in Media Containing Colchicine.

A. W. WALKER AND GUY P. YOUMANS.

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Colchicine is toxic for plant and animal cells, and seems to be specifically a mitotic poison, preventing normal division of the chromosomes and thus causing mutation.¹ True bacterial cells differ from the cells of the higher plants and animals, in that so far as is known they do not contain chromosomes or well defined nuclei.

If the above statements are true then we might expect that colchicine would have either no effect or a different effect on bacterial cells than on the cells of the higher plants.

This work was designed to study the effect of colchicine on several different types and species of bacteria with respect to the character of growth, colony characteristics, cell morphology, biochemical reactions, and acceleration or inhibition of multiplication of the organisms.

Methods. A 4% solution of colchicine in plain meat infusion broth was sterilized by filtration through a Berkefeld N filter. This solution of colchicine was then diluted with broth to the desired concentrations. All media used were adjusted to pH 7.2.

Serial Transfers in Plain Broth and Colchicine Broth. One standard 4-mm loopful of a 24-hour broth culture of Staphylococcus H was inoculated into 5 cc of a 2% colchicine broth and into the same amount of plain broth. A culture of B. typhosus 109 was inoculated in the same manner. All cultures were incubated at 37°C. Serial transfers were made daily into fresh media of the same kind (colchicine into colchicine, and plain broth into plain broth) for 12 days and every 48 hours thereafter until a total period of 22 days had elapsed.

At the time of each transfer subcultures were made on plain agar plates in order to observe colony morphology and also smears made and stained by Gram's method. On the 8th and 16th transfer all the cultures were inoculated into various carbohydrate media, milk and gelatin for observations of their biochemical reactions.

Old Cultures. Cultures of Staphylococcus aureus H and B.

¹ Nebel, B. R., and Ruttle, M. L., J. Heredity, 1938, 29, 2.

typhosus 109 were made in 2% colchicine and plain broth as before but instead of serial transfers the original cultures were incubated at 37°C for 22 days during which time subcultures were made every 4 days and treated in the same manner as described above.

Scrial Transfers on Plain and Colchicine Agar. Plain agar and 2% colchicine agar plates were inoculated with a 24-hour broth culture of Staphylococcus aureus H and B. typhosus 109 in such a manner as to obtain isolated colonies. After 48 hours' incubation single colonies were picked and re-streaked on fresh plates. The colonies from the colchicine agar plates were streaked on colchicine agar and also on plain agar plates, and the colonies from the plain agar plates were streaked on plain agar and also on colchicine agar plates. These transfers were made every 48 hours over a total period of 22 days. Smears were made of the colonies and stained by Gram's method.

Other Organisms. A second series of colchicine broth tubes was inoculated using in this series varying concentrations of colchicine (2%, 1%, 0.50% and 0.25%). The organisms used were Staphylococcus aureus 1038. Staphylococcus aureus L, Streptococcus hemolyticus, M. catarrhalis and B. megatherium. Control tubes of plain broth were inoculated at the same time. Incubation was at 37°C for 22 days with the following observation at 48-hour intervals for the first 6 days and at 4-day intervals thereafter. Subcultures were made on plain agar plates for observations of colonies, smears made and stained by Gram's for morphology, and inoculation into various carbohydrate media, gelatin and milk to note changes, if any, in cultural reactions Comparison was made of the amount of growth in the colchicine broth with that in plain broth controls as an indication of any inhibiting or accelerating action of the colchicine on the growth of the organism.

Results. The type of growth of Stapylococcus aureus H in 2% colchicine broth was markedly different, being a coarse granular growth which settled out rapidly, in contrast to the diffuse turbid growth of the plain broth controls. On 2% colchicine agar the colonies were wrinkled, waxy, and of a peculiar greenish-yellow color. The cell morphology in the colchicine broth and agar differed from the controls in that the organisms were larger and arranged in tetrads and had the appearance of a stained preparation of sarcinæ. These changes in growth, colony and cell morphology occurred on the first transfer to media containing colchicine and could be obtained only when grown in the presence of colchicine. On transfer to plain media the organisms immediately reverted to normal.

There was no change in the biochemical reactions. All the other organisms used, including 2 other strains of Staphylococcus, showed no variation from normal when grown in the presence of colchicine. The growth of Streptococcus hemolyticus was inhibited by 1% of colchicine; 0.50% and 0.25% had no apparent effect. There was marked but not complete inhibition of growth of M. catarrhalis in 2% colchicine, some inhibition in 1% but none in 0.50% and 0.25%. The staphylococci and B. megatherium grew equally well in all concentrations of colchicine used. There was no evidence of any stimulating effect on the growth of any of the organisms.

Blakeslee² says that colchicine affects only the cell division of the chlorophyl-bearing plants and that the fungi including the bacteria are not affected. Jennison³ could detect no change in the rate of reproduction or colony morphology of bacteria in the presence of colchicine. With the exception of one strain of Staphylococcus which showed a temporary variation in cell and colony morphology when grown in the presence of colchicine, our results are in accord with these observations but further indicate that colchicine does not affect the cell metabolism of bacteria.

11428

The Liver and Endogenous Androgens.*

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Medical School, Chicago.

It is well known that most androgens and estrogens are either inactive when administered orally, or are less potent than when administered subcutaneously. In the case of the estrogens, experimental evidence has been offered to explain this phenomenon. All workers agree that the lack of effectiveness with oral administration is due to inactivation of estrogens in the liver. When natural estrogens are incubated in vitro with liver, 1,2 their potency is lost.

² Blakeslee, A. F., Science, 1939, 89, 10.

³ Jennison, M. W., J. Bact., 1940, 39, 20.

^{*} Supported in part by a grant from the Josiah Maey, Jr., Foundation.

Zondek, B., Skand. Arch. f. Phys., 1934, 70, 133.
 Heller, C. G., Endocrin., 1940, 26, 619.

When ovaries are implanted intra-mesenterically so that the venous drainage is through the liver, the animal manifests changes characteristic of castration but, when the same ovary is later regrafted into the axillary region, the castration effects disappear.³ Talbot⁴ showed that impairment of liver function by CCl₄ apparently increased the concentration of endogenous estrogen in the blood stream as evidenced by increase in uterine weight.

Concerning the possible inactivation of androgens by the liver, only two reports have been offered. Biskind and Mark⁵ showed that pellets of testosterone propionate are ineffectual when implanted into the intact spleen, but not when implanted into the spleen after the original blood supply of this organ has been altered. This indicates that inactivation of the testosterone propionate occurs only when it passes from the site of absorption directly through the portal system. Biskind,⁶ by the same type of experiment, showed that methyl testosterone is also inactivated when it passes first through the portal system. In view of the known oral effectiveness of methyl testosterone, in contrast to all other androgens, it seems probable that this substance is removed from the gastro-intestinal tract by some route other than the portal system.

The present report is concerned with the possible rôle of the liver in the inactivation of testicular androgens. The transplantation method devised by Golden and Sevringhaus was used.

Twenty-five males, 20 to 23 days old, were castrated and, in each case, one-half of a testis was sutured into the gastro-splenic mesentery. At the same time 21 males of the same age were castrated and one-half a testis was implanted subcutaneously into the groin of each.

One or two months after implantation the surviving animals were killed. In each case the size and appearance of the penis was noted, the general character and vascular relationships of the implant were established, and the ventral prostates and seminal vesicles were removed and weighed. In some of the animals the thymus was also weighed. It was thought that the thymus might present an additional check for the presence or absence of androgens, inasmuch as thymic hypertrophy after castration has been mentioned in the literature.

³ Golden, June B., and Sevringhaus, E. L., Proc. Soc. Exp. Biol. And Med., 1938, 39, 361.

⁴ Talbot, N. B., Endocrin., 1939, 25, 601.

⁵ Biskind, G. R., and Mark, Jerome, Bull. Johns Hopkins Hospital, 1939, 65, 212.

⁶ Biskind, G. R., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 259.

Part of the graft tissue from each animal was preserved in Bouin's

With a few exceptions, the findings were fairly uniform. In almost all cases the implanted tissue appeared to be fairly healthy, seminiferous tubules were visible under the dissecting microscope. In the intra-mesenteric location, the grafted tissue was usually spread out over a considerable area, closely associated with the pancreas, so that the tubules in these grafts were somewhat more dispersed than in the subcutaneous grafts.

The animals with intra-mesenteric implants gave no evidence of androgenic stimulation. The penis was small, poorly developed and barely evertable; the prostates and seminal vesicles were small and atrophic in appearance. The animals with subcutaneous implants, however, showed some degree of androgenic stimulation in that the penis was generally normal in size and structure and the prostates and seminal vesicles were well above the castrate level in weight.

The weight findings in those animals having healthy implants are recorded in Table I. In the first set of experiments, the number of animals was small, therefore no attempt at statistical analysis was made. In the second set of experiments the prostate and seminal vesicle weights of the subcutaneous group were significantly larger than those of the intra-mesenteric group. The thymus weights were not significantly different.

The implants from all animals were sectioned and examined microscopically. In most cases the implanted tissue appeared to be well established and well vascularized. Regions of lymphocytic

TABLE I.
Prostate and Seminal Vesicle Weights in Rats with Testicular Implants.

Situation of implants	No. operated	to	I No. with healthy implants	body	prost.	G	Avg S.V. wt	g .	Avg thymus wt	G
	Operated	at 22-23	days of ag	e. Ir	nplants	resid	ent fo	r 2 mo	onths.	
Intra-										
mesen.	10	7	5	213	4.92		5.93			
Subcut.	10	4	4	187	29.37		29.37			
Intra-	Operated	at 20 d	lays of age	e. In	aplants	resid	lent fo	r 1 m	onth.	
mesen.	15	13	12	146	5.26	0.29	7.03	0.26	509.6	31.9
Subcut.	11	11	10		23.77				433.9	45.0

σ derived by the formula
$$\sqrt{\frac{\Sigma \delta^2}{n(n-2)}}$$

Significant difference > $3\sqrt{(\sigma_1)^2 + (\sigma_2)^2}$

infiltration and fibrosis were observed. The seminiferous tubules which were found in all healthy implants resembled those of experimental cryptorchid testes in that generally Sertoli cells alone were present, although occasionally a few spermatogonia were observed. Interstitial cells were present in all implants. The number of interstitial cells varied from a few scattered cells between the tubules to fairly large areas of densely packed cells. The latter condition may be regarded as true interstitial cell hypertrophy. Generally the grafts which were in situ for 2 months exhibited better development of interstitial cells than those which were only one month old.

One fact has been shown by these experiments. If viable testicular tissue is situated so that its venous drainage passes directly through the liver, there is no evidence of androgenic stimulation or maintenance in the accessory structures. If, however, the testicular tissue is located so that it is associated with the peripheral circulation, there is some androgenic activity registered in the accessories. The conclusion that the liver is responsible for the lack of androgenic effect in the case of the intra-mesenteric grafts is valid only if it can be shown that the graft tissue in this location is actually producing androgens. Jeffries⁷ and others have shown that testes rendered cryptorchid continue to produce androgens, in normal or nearly normal amounts, for a considerable period, even when the germinal elements are completely degenerate. In the grafted tissues from the animals reported here, interstitial cells were present and, in some cases, hypertrophied. This finding cannot be regarded as absolute proof of secretory function in the grafted tissue, but is strongly indicative of such function.

Summary. When testicular tissue was implanted into the immature rat so that its venous drainage passed through the liver, there was a lack of androgenic stimulation evident in the castrate condition of the penis, prostate and seminal vesicles after a period of one to 2 months. When the testicular tissue was implanted subcutaneously, some androgenic effect on the penis and accessories was obtained. Interstitial cells in varying numbers were present in all grafts. This finding indicates probable endocrine function by the implanted tissue. It is tentatively concluded that the lack of androgenic stimulation in animals with intra-mesenteric implants of testicular tissue is due to inactivation of testicular androgens by the liver. This is in agreement with similar experiments using implanted crystals of testosterone propionate and methyl testosterone. 5,6

⁷ Jeffries M. E., Anat. Rcc., 1931, 48, 131.

11429

Antiserum for Renin.*

C. A. JOHNSON AND G. E. WAKERLIN.

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In some earlier experiments on renin tachyphylaxis in the dog.¹ it was occasionally noticed that when an animal had become tolerant to dog renin is would still give a pressor response to the injection of rabbit renin. This observation, in the light of increasing evidence to support the protein-like character of renin, suggested the possibility of a species difference in this pressor substance as extracted from the renal cortex of these animals. Consequently, the idea occurred to us that a study of the immune responses to renin may furnish a new experimental approach to elucidate further its nature and properties. Even if renin is a hormone, as has been suggested, it may still produce recognizable immune or antihormone responses equally valuable for our immediate purpose.²

This report is concerned with the preparation and properties of an antiserum produced in rabbits injected with pressor active extracts of the renal cortex of the dog. A preliminary observation on the pressor negating effect of serum from a dog receiving injections of hog renin is also recorded

The method used for the extraction of renin was essentially that described by Grossman.³ Dry, powdered, cortical residue, left after alcohol or acetone extraction, was used as crude material from which extracts of the pressor active principle were made. These (extracts) were further purified to remove much of the associated protein. About 30 cc of this partially purified extract was mixed with an equal volume of colloidal aluminum hydroxide. The colloidal aluminum hydroxide with the proteins, including renin, adsorbed on it was concentrated to half the volume by centrifuging and this gelatinous mixture injected intramuscularly into one rabbit.⁴ At the end of 2 or 3 weeks the rabbits were bled to ascertain the presence of antibodies in the serum. If antibodies in appreciable titer were present

^{*} This work was aided by a grant from the Graduate School Research Fund of the University of Illinois.

¹ Wakerlin, G. E., and Johnson, C. A., Proc. Am. Phys. Soc., 1940, p. 192.

² Collip, J. B., Ann. Int. Med., 1934, 8, 10.

³ Grossman, E. B., Proc. Soc. Exp. Biol. and Med., 1938, 39, 40.

⁴ Hektoen, L., and Welker, W. H., J. Infect. Dis., 1933, 53. 309.

the animal was bled to death and the antiserum preserved in the refrigerator. If a low titer serum was found several intravenous injections of the original extract were given to augment its titer. Immunization may also be accomplished by the more common method of injecting the soluble antigen (renin) intravenously in increasing amounts at 3-day intervals.

Since the antigen used was a mixture of renin, kidney protein(s), and serum proteins (especially pseudoglobulin), precipitins for the last were demonstrable in the antiserum. Precipitins for dog serum pseudoglobulin in the antiserum could be removed by in vitro adsorption without affecting the renin antibody. To demonstrate the presence of an antibody for renin we had to depend on the bioassay of mixtures of the antiserum and renin. Such assays were always controlled in the same animal by a similar dose of renin mixed with normal rabbit serum. After a number of attempts to determine the optimum quantity relationship between antiserum and renin we routinely mixed 2 volumes of antiserum with one volume of renin and allowed these mixtures to remain at 4°C at least overnight.

Dogs were used for all assays. The femoral artery was cannulated under light ether or local (procaine) anesthesia and the blood pressure recorded with a mercury manometer. Some of the dogs were also subjected to bilateral nephrectomy. After recovery from these operative procedures, the animal was given an initial dose of renin intravenously, usually equivalent to one-half gram of

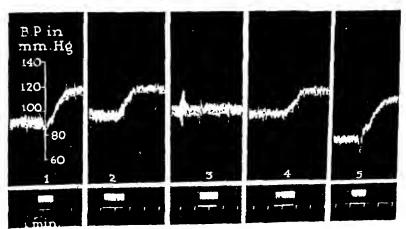


Fig. 1.

Dog, 7 kg, male, bilaterally nephrectomized. 1 Initial dose (2 ee) of dog renin injected, 2 two ee dog renin mixed with four ec normal rabbit serum, 3 two ce dog renin mixed with four ee antiserum for dog renin, 4 repeat of 2, 5 repeat of 1.

kidney per kilo of body weight, to establish the degree of pressor response. When the blood pressure had again assumed its original level a control dose of renin mixed with normal rabbit serum was injected. Figs. 1 and 2 illustrate results which are typical of those obtained by the assay of antiserum from 4 rabbits on a total of 15 dogs.

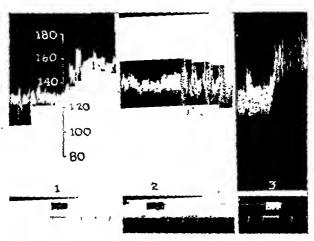


Fig. 2.

Dog, 8 kg, female, not nephrectomized. 1 One part (2 cc) dog renin mixed with two parts normal rabbit serum, 2 one part dog renin mixed with two parts antiserum for dog renin, 3 repeat of 1.

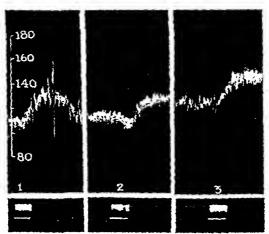


Fig. 3.

Dog, 20 kg, male, not nephrectomized. 1 One part (5 cc) rabbit renin mixed with two parts normal rabbit serum, 2 one part rabbit renin mixed with two parts antiserum for dog renin, 3 repeat of 1.

In 4 dogs we observed the effect of the injection of mixtures of rabbit renin and the antiserums for dog renin just described. The neutralizing effect of these antiserums on the pressor response to rabbit renin was distinctly less than it was for dog renin, as illustrated by Fig. 3. These results suggest a partial antigenic similarity between dog and rabbit renins although further work is necessary to prove this point conclusively.

Since pituitrin has a pressor effect similar to renin on the peripheral vascular bed it was of interest to determine whether the antiserum had any effect on the pressor response to pituitrin. Comparing injections 4 and 5 in Fig. 4, it is apparent that the mixing of pituitrin and antiserum did not change the character or degree of response to this agent as determined on 2 dogs.

In connection with another phase of our study of renin we had occasion to examine the serum of a dog which had received daily intramuscular injections of hog renin in a dosage of one gram of kidney equivalent per kilo for a period of 12 weeks. When serum from this dog was mixed with hog renin and injected into 3 assay dogs, a decidedly diminished response was observed (Fig. 5). No precipitins for hog serum proteins were demonstrable in this antiserum.

The neutralizing substance for renin demonstrated by these results may prove useful in throwing light on the question of whether renin is the pathogenetic agent in experimental renal ischemic hyper-

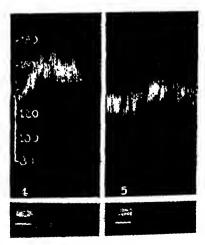


Fig. 4.

Dog, (same as in Fig. III.). 4 Three units pituitrin mixed with four ee antiserum for dog renin, 5 three units pituitrin mixed with four ee normal rabbit serum.

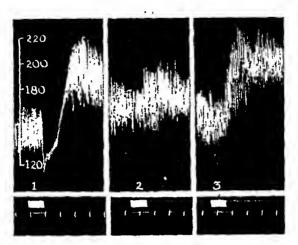


FIG. 5.

Dog, 7 kg, male, not nephrectomized. 1 One part (2 ec) hog renin mixed with two parts normal dog serum, 2 one part hog renin mixed with two parts serum from dog which had received injections of hog renin, 3 repeat 1.

tension. Speculating further, such an "antirenin" may conceivably have therapeutic value, when passively administered or actively produced in experimental renal ischemic hypertension. Experiments aimed at elucidating these possibilities, as well as a study of the immune responses to renins of other species, are now in progress.

Conclusions. 1. The rabbit is able to produce an antiserum which counteracts the pressor effect of dog renin. 2. The antiserum for dog renin appears to diminish the pressor response to rabbit renin. 3. This antiserum has no effect on the pressor action of pituitrin. 4. The dog appears able to produce an antiserum to hog renin. 5. The active principle of the antiserums (antirenin) is most likely a non-precipitating antibody or possibly an antihormone. 6. Studies are now in progress to determine the value of antirenin in the therapy of experimental renal hypertension and also in the elucidation of the possible pathogenetic rôle of renin in this form of hypertension.

11430

Tissue Culture Growth Stimulants from Ground Frozen-Dried Chick Embryos.

Duncan C. Hetherington and Jane Stanley Craig. (Introduced by F. H. Swett.)

From the Department of Anatomy, Duke University School of Medicine, Durham, N. C.

It has been demonstrated that frozen-dried plasma and similarly treated embryo juice used in combination, after solution in distilled water, form an adequate and satisfactory medium for the growth of tissue cultures. Further experimentation with such preparations has brought forth evidence that a much more potent embryo juice could be secured if the embryos themselves were ground and frozen-dried before extraction,

Eleven-day chick embryos were removed from their shells and membranes and reduced to a gray, grumous mass with sea sand in a Ten Broeck grinder. The material was pipetted off and allowed to stand in a container for a short while to permit the larger particles of grit to settle. Later the ground substance was introduced into pyrex ampoules, frozen-dried and sealed in vacuo by means of a Lyophile² apparatus. All manipulations were carried out aseptically.

The dried matter at first was crusty but could be reduced to a powder by forcibly shaking or by tapping the containers. The ampoules were stored at room temperature for 14 months before any tests were instituted. A preliminary series of cultures was made with embryo juice prepared by steeping a portion of the dried powder in Ringer-Tyrode's solution in an ice box prior to centrifuging. The cultures grew so exuberantly that it was decided to test statistically the growth-promoting effect of this extract on cultures of embryo chick heart.

By calculation from fresh embryos dried to constant weight in an oven 70 mg of dry powder were equivalent to one gram of fresh embryo. This figure was only approximately accurate since there was no simple way of determining how much sand and glass dust might have been retained in the powder. However, 20% embryo juice was made by adding 70 mg of powder for each 5 cc of Ringer-Tyrode's solution. The mixture was agitated gently, steeped for

¹ Hetherington, Duncan C., and Craig, Jane Stanley, Proc. Soc. Exp. Biol. and Med., 1939, 42, 831.

² Flossdorf, Earl W., and Mudd, Stuart, J. Immunol., 1935, 29, 389.

TABLE I.

		Control Series	Series		 					Experimental Series	al Series	
Days of growth	Mean total area in mm2	Probable error of mean	6	Total growth in %	D_{M}	$\mathfrak{F}_{\mathrm{D}}$	$3\sigma_{\mathrm{D}}$	Significant difference	Mean total area in mm²	Probable error of mean	ь	Total growth in %
0	0.77	+0.03	0.37	0	0.13	70.0	0.12	+	0.64	±0.03	0.31	0
, , , ,	1,45	+0.05	0.65	88	0.04	0.11	0.33	0	1,49	10.0€	0.81	133
¢1	2,51	±0.07	1.12	995	0.35	0.19	0.57	0	2.83	±0.10	1.46	343
က	4,33	±0.17	6. 14.01	462	1.75	0.40	1.38	+	80.9	+0.28	3.92	850
-) 1	7.28	140.33	3.17	845	2.48	0.71	2.13	+	9.76	+0.38	5.47	1425
ເລ	8.61	+0.32	4.53	1018	4.36	0.77	2.31	+	12.97	1+0.40	5.74	1926
9	11.26	+0.38	5.33	1362	5.56	0.85	2,55	-+-	16.82	±0.43	6.08	2528
2	14.06	110.39	5.59	1726	5.05	0.91	2.73	+	19.08	十0.47	6.53	2881
00	17.54	1+0.40	5.66	2178	5.84	0.88	5.64	+	23.38	₩0.43	6.14	3553
							-					

 $D_{M'}$ difference of the means; $\sigma_{D'}$ standard error of the difference of the means; significant difference $=D_{M}>3\sigma_{D'}$

11430

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¹ Hetherington, Duncan C., and Craig, Jane Stanley, Proc. Soc. Exp. Biol. AND Med., 1939, 42, 831.

² Flossdorf, Earl W., and Mudd, Stuart, J. Immunol., 1935, 29, 389.

growth increase in the experimental series, indicating rather conclusively the greater potency of the embryo-juice. Furthermore, careful microscopic examination of the living cells indicated that those in the test series did not develop fat droplets as readily as the controls and at all times appeared in better condition.

Fowler³ in a series of titrations of embryo juices upon chick heart fibroblasts concluded that best growth was obtained from utilizing extracts from 11- to 14-day whole embryos since during that growth period greater morphological and physiological changes took place within the developing animal—attributed possibly to endocrine production.

The potency of embryo juice prepared by the present method may be ascribed to increased accessibility of the cell contents to extraction and solution by the Ringer-Tyrode's saline. The preliminary grinding reduced the tissues to smaller particles than could be accomplished by mere mincing and the subsequent freezing* and drying disrupted most of the cells. In consequence many more substances from all parts of the embryo went into solution, probably in an undenatured condition.^{2, 4}

The experiment reported has shown that a very excellent embryo juice may be prepared from ground frozen-dried chick embryos. The dried powder retained its potency for 14 months and frozen-dried embryo juice derived from it was extremely active after 3 months' additional storage. There seems hence to be no reason why such products as frozen-dried embryo powder and frozen-dried plasma could not be used to considerable advantage in tissue culture laboratories.

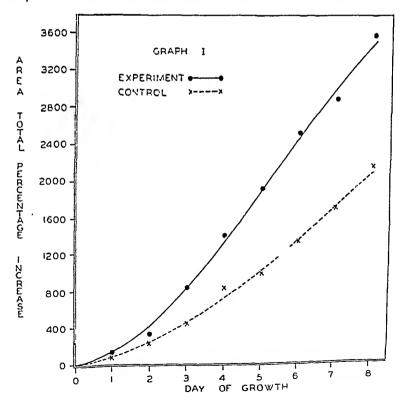
³ Fowler, Ona M., J. Exp. Zool., 1937, 76, 235.

^{*} Neutral red spreads made from tissues of chick embryos frozen to -70°C and then thawed rapidly at 37°C showed that very few of the cells remained intact. The nuclei alone appeared to be unbroken.

⁴ Elser, William J., Thomas, Ruth A., and Steffin, Gustav I., J. Immunol., 1935, 28, 433.

24 hours in an ice-box and centrifuged. The resultant slightly pink, opalescent fluid was removed and delivered in 1 cc quantities into ampoules and frozen-dried. Again aseptic technic was employed throughout. After 3 months storage this frozen-dried embryo juice made from the frozen-dried embryos was tested for growth-promoting properties. Two series of tissue cultures were planted from 11-day chick embryo hearts. At any one planting the same heart was used for both the experimental and the control cultures. The former were planted in equal parts of the new type embryo juice and frozen-dried plasma (stored for 6 months); while the latter were similarly treated except that 20% juice from fresh 11-day embryos was employed.

Delineascope and planimeter records were kept of the areas of the original explants; thereafter for 8 days, the total area of each culture was measured at 24-hour intervals. The results obtained from 90 cultures in each series of the experiment appear in Table I and Graph 1. From these it will be seen that there was a significant



growth increase in the experimental series, indicating rather conclusively the greater potency of the embryo-juice. Furthermore, careful microscopic examination of the living cells indicated that those in the test series did not develop fat droplets as readily as the controls and at all times appeared in better condition.

Fowler³ in a series of titrations of embryo juices upon chick heart fibroblasts concluded that best growth was obtained from utilizing extracts from 11- to 14-day whole embryos since during that growth period greater morphological and physiological changes took place within the developing animal—attributed possibly to endocrine production.

The potency of embryo juice prepared by the present method may be ascribed to increased accessibility of the cell contents to extraction and solution by the Ringer-Tyrode's saline. The preliminary grinding reduced the tissues to smaller particles than could be accomplished by mere mincing and the subsequent freezing* and drying disrupted most of the cells. In consequence many more substances from all parts of the embryo went into solution, probably in an undenatured condition.^{2, 4}

The experiment reported has shown that a very excellent embryo juice may be prepared from ground frozen-dried chick embryos. The dried powder retained its potency for 14 months and frozen-dried embryo juice derived from it was extremely active after 3 months' additional storage. There seems hence to be no reason why such products as frozen-dried embryo powder and frozen-dried plasma could not be used to considerable advantage in tissue culture laboratories.

³ Fowler, Ona M., J. Exp. Zool., 1937, 76, 235.

^{*} Neutral red spreads made from tissues of chick embryos frozen to -70°C and then thawed rapidly at 37°C showed that very few of the cells remained intact. The nuclei alone appeared to be unbroken.

⁴ Elser, William J., Thomas, Ruth A., and Steffin, Gustav I., J. Immunol., 1935, 28, 433.

11431 P

Behavior of Pigment Cells from Cultures of Neural Crest When -Grafted Back into the Embryo.*

Frances Dorris. (Introduced by R. G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

Neural crest taken from chick embryos in early somite stages produces large numbers of pigment cells both *in vitro*, and in grafts to the limb buds of older host embryos, the melanophores later appearing in the dermis and feathers of the hosts. This work has been corroborated by Eastlick, who transplanted limb buds with or without the neural crest, and found that the appearance of pigment was correlated with the presence of the neural component in the grafts.

The earlier cultures were short time cover slip preparations designed to test the capacity for differentiation of the tissue explanted. In view of the complex prospective potency of the neural crest in the embryo, it seemed advisable to attempt to develop pure cell strains in order to study the behavior of these cells under controlled conditions. For the past year, explants of early somite neural crest from colored breeds have been grown in small culture flasks in a standard medium of plasma and embryonic extract, according to the technic developed by Carrel and his associates. Such cultures grow rapidly during the first week in vitro, soon producing a ring of dark brown or black cells. Growth then continues at a slower rate, and strains of cells are produced which are uniform in appearance and behavior, being composed of typical branched melanophores which spread out upon the clot or become aggregated in clumps.

Cells from these cultures have been grafted to embryos of another breed than that of the donor, a single culture furnishing enough tissue to graft to as many as 30 host embryos, without entirely exhausting the original strain. When a graft is inserted into the limb bud of a white host (which is subsequently observed through a transparent window in the shell), the ectoderm heals over the tissue,

^{*}This research was supported by a grant from the Rockefeller Foundation to Yale University for work in experimental embryology under the direction of Professor Ross G. Harrison.

¹ Dorris, F., Proc. Soc. Exp. Biol. and Med., 1936, 34, 448; Roux Arch., 1938, 138, 323.

² Dorris, F., Anat. Rec., 1938, 70, 91; J. Exp. Zool., 1939, 80, 315.

³ Eastlick, H. L., Collecting Net, 1938, 13, 151; Genetics, 1939, 24, 98; Anat. Rec., 1939 a., 73, Suppl. 2, 64; J. Exp. Zool., 1939 b., 82, 131.

which is still clearly visible as a dark mass below the surface. During the first 2 days, migration of melanophores from the graft occurs, and these are finally found in the dermis, epidermis, and developing feathers of the femoral tract in host embryos fixed at the 12th to 15th day of incubation. A total of 145 grafts have been made, the tissue being furnished by pure strain cultures of differentiated melanophores obtained from the neural crest of Australorp embryos (a Black Orpington breed), the hosts being White Leghorn or Rhode Island Red embryos ranging in age from 3 to 5 days of incubation. The work, which is being extended to include other breeds of fowl, will be reported fully elsewhere.

Two points brought out by these experiments are of special interest. One is the maintenance in grafts, of the slow growth rate established in vitro. In striking contrast to the behavior of neural crest grafted directly from the embryo, which produces pigmented areas covering the whole leg, shank, and foot, as well as parts of the dorsal and ventral trunk regions, neural crest melanophores from cul-, tures formed patches of pigment identical in appearance, but very much smaller in extent, never involving even the greater part of the femoral feather tract. Even more interesting is the fact that these cells, although transplanted into an environment composed of embryonic tissues physiologically younger, and thus forming what has been described as an "embryonic field", are apparently unaffected, and continue to reproduce as pigmented cells, behaving in a manner quite similar to that seen in vitro. This stability of both differentiation and growth rate in the formed tissue, is in striking contrast to the extreme lability of the embryonic region from which the cells were originally derived.

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¹ Dorris, F., Proc. Soc. Exp. Biol. and Med., 1936, 34, 448; Roux Arch., 1938, 138, 323.

² Dorris, F., Anot. Rec., 1938, 70, 91; J. Exp. Zool., 1939, 80, 315.

³ Eastlick, H. L., Collecting Net, 1938, 13, 151; Genetics, 1939, 24, 98; Anat. Rec., 1939 a., 73, Suppl. 2, 64; J. Exp. Zool., 1939 b., 82, 131.

1 mg O.T. All 7 died within 12 to 37 days after infection and showed extensive parenchymatous tuberculosis with caseation. It was interesting to note that 5 of these guinea pigs had miliary tuberculosis of the kidneys, a condition not encountered with subcutaneous or intraäbdominal infection.

Albino Rats: All of these rats were negative to intracutaneous doses of 1 mg O.T., and tolerated 500 mg of O.T., injected subcutaneously, without noticeable symptoms. At necropsy, 46 days after infection, 3 of 4 animals showed a sparse scattering of tubercles in the lungs, and some suspicious areas in the spleen. No other macroscopic evidence of tuberculosis was found. The 3 remaining animals were sacrificed 65 days after infection, subsequent to unsuccessful attempts at shocking them with 1 g O.T. These rats showed the same scant tuberculous involvement as those sacrificed earlier.

Cotton Rats: All of the cotton rats reacted negatively to the intracutaneous injection of 1 mg O.T. 2 weeks after infection. All 4 animals which had been sacrificed at 46 days showed miliary tuberculosis of the lungs, spleen, and lymph glands. One cotton rat had miliary tuberculosis of the liver, and 3 showed tubercles in the kidneys. In all 4 cotton rats definite tissue-destruction and caseation was observed at the site of infection.

Of the 3 surviving animals, one died 65 days after infection, and showed extensive tuberculosis in the lungs, liver, and kidneys. The 2 remaining cotton rats were given 250 mg O.T. subcutaneously at the same time. One died the following day with no evidence of tuberculin shock. The other cotton rat showed no symptoms. It was then injected subcutaneously with 500 mg O.T. No evidence of tuberculin-intoxication was observed during the next 24 hours. The same animal was then injected intraäbdominally with 1 g O.T. Within 5 minutes after injection, the animal became restless and dyspneic, and died within 40 minutes. At necropsy, there was serous fluid in the thoracic and abdominal cavities, and focal hemorrhages were found about the tubercles in the various organs. Both of the last 2 cotton rats showed miliary tuberculosis of the lungs, liver, spleen, glands, and kidneys.

Histopathology: Histological study of the tissues confirmed and further emphasized the differences found at autopsy. The tubercles in the cotton rat are predominantly epithelioid in type, some showing caseation, but no giant cells were observed. In most sections the tubercles were conglomerated. Acid-fast bacilli are easily demonstrated in the tubercles. A more complete description of the histopathology will be given in a later communication.

Summary and Conclusions. A comparative study of the Eastern

11432 P

Experimental Tuberculosis in the Cotton Rat (Sigmodon hispidus littoralis).*

M. MAXIM STEINBACH AND CHARLES J. DUCA.

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Armstrong¹ was the first to discover that the Eastern cotton rat differs from other rodents in that it proved susceptible to the virus of poliomyelitis. Similar results were recently obtained by Jungeblut and Sanders² with another strain of monkey poliomyelitis virus. Since then there has been carried out in this department a systematic study of the reaction of cotton rats to other infections and toxic agents. It was found that this animal (Sigmodon hispidus littoralis) resembles the guinea pig in its susceptibility to diphtheritic toxin and bacillary infection with C. diphtheriæ.³ This resemblance between the two species was also shown to exist with respect to their susceptibility to infection with Trypanosoma equiperdum.⁴ It seemed of interest to determine how this animal compares with the guinea pig and the albino rat in its susceptibility to infection with tubercle bacilli.

Experimental Methods. Seven albino rats (averaging 100 g in weight), 7 cotton rats (averaging 80 g), and 7 guinea pigs (averaging 350 g) were tested intracutaneously with 1 mg O.T. and found to be negative. All animals were then infected intravenously with 1 mg of bovine tuberele bacilli (B1). In some instances part of the inoculum escaped into the surrounding tissue as evidenced by the occurrence of infection of the regional glands. Two weeks after infection all animals in the 3 groups were again tested intracutaneously with 1 mg O.T. Half of the survivors in each group were sacrificed for pathological studies 46 days following infection; at the end of 2 months the remaining animals were tested for susceptibility to tuberculin-shock.

Results. Guinea Pigs: Six animals which survived longer than 2 weeks were found to be highly sensitive to intracutaneous doses of

^{*} Aided by grants from the National Tuberculosis Association and the Philip Hanson Hiss, Jr., Memorial Fund.

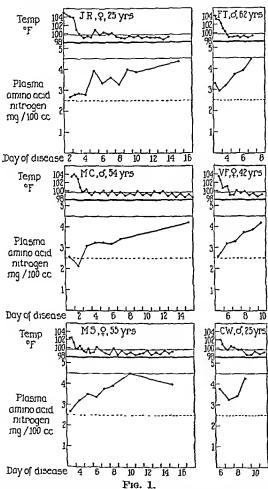
¹ Armstrong, C., Pub. Health Rep., 1939, 34, 1719.

² Jungeblut, C. W., and Sanders, M., Proc. Soc. Exp. Biol. And Med., 1940, 44, in press.

³ Jungeblut, C. W., Proc. Soc. Exp. Biol. and Med., 1940, 43, 479.

⁴ Culbertson, J. T., J. Parasit., in press.

alated, and immediately centrifuged. The plasma was pipetted off and the sample kept in the icebox until the analyses could be run. Plasma amino acid nitrogen was determined by the ninhydrin-CO₂ method of Van Slyke and Dillon³ as applied to blood by MacFadyen and Van Slyke.⁴ Farr and MacFadyen¹ have already pointed out that



Graphie summary of results of serial plasma amino acid nitrogen determinations on 6 patients with pneumococcal pneumonia. The average normal value for plasma amino acid nitrogen is 4.50 mg per 100 cc. The critical value for nephrotic crises is 2.5 mg per 100 cc as indicated by the broken line. Note the pronounced hypoaminoacidemia during the acute phase of the disease followed by a rise during recovery. Temperature charts are included to briefly summarize the clinical course.

³ Van Slyke, D. D., and Dillon, R. T., Compt. rend. Lab. Carlsberg, 1938, 22. 480.

⁴ MaeFadyen, D. A., and Van Slyke, D. D., in preparation.

cotton rat, the albino rat, and the guinea pig suggests that the cotton rat occupies a position midway between the other two species regarding its susceptibility to infection with pathogenic bovine tubercle baeilli. It is definitely more susceptible than the naturally resistant albino rat but not as highly susceptible as the guinea pig. Like the albino rat, the tuberculous cotton rat is insensitive to skin-test doses of tuberculin, and tolerates large amounts injected subcutaneously. Since the observations on tuherculin shock are based upon findings obtained from only one animal, further work is necessary to confirm this point.

11433 P

Hypoaminoacidemia in Patients with Pneumococcal Pneumonia.

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The observations by Farr and MacFadyen¹ and Farr² on the incidence and duration of hypoaminoacidemia in children with the nephrotic syndrome have reopened the question of the constancy of blood amino acid concentration in other disease states. Because of certain clinical similarities between the onset of recovery in pneumococcal pneumonia and that of children from nephrotic crises, as well as the prevalence of pneumococcal infections in nephrotic children, we believed that extension of studies on blood amino acids to diseases other than Bright's disease might profitably begin with pneumonia.

Studies of plasma amino acids on all patients admitted to the pneumonia service in this hospital have been made. While these are not yet completed, the results thus far have been sufficiently striking

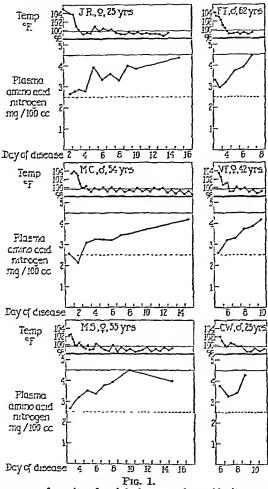
and uniform to warrant reporting them.

Methods. Blood was drawn from each patient immediately upon admission to the hospital and before any therapy was begin. Subsequently, blood was drawn at selected intervals, when possible after an overnight fast, otherwise after an interval of at least 4 hours had elapsed from the time when the last protein-containing food was given. The blood was drawn with care to prevent hemolysis, ox-

¹ Farr, L. E., and MacFadyen, D. A., Am. J. Dis. Child., 1940, 59, 782.

² Farr, L. E., J. Ped., in press.

alated, and immediately centrifuged. The plasma was pipetted off and the sample kept in the icebox until the analyses could be run. Plasma amino acid nitrogen was determined by the ninhydrin-CO₂ method of Van Slyke and Dillon³ as applied to blood by MacFadyen and Van Slyke.⁴ Farr and MacFadyen¹ have already pointed out that



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³ Van Slyke, D. D., and Dillon, R. T., Compt. rend. Lab. Carlsberg, 1938, 22. 480.

⁴ MacFadyen, D. A., and Van Slyke, D. D., in preparation.

even the Van Slyke nitrons acid method may be inadequate to permit detection and interpretation of small changes in plasma amino acid levels. For this reason the ninhydrin-CO₂ method was used in this study because of its greater specificity.

Results. The results on 6 patients are summarized graphically in Fig. 1. As early as the first day of disease a distinct drop in the plasma amino acid nitrogen had occurred. With recovery from the pneumonia all patients showed a rise of the plasma amino acid nitrogen to normal levels. Whereas the average concentration of plasma amino acid nitrogen in nephrotic children was about 3 mg % which decreased to as little as 1.2 mg % during nephrotic crises, 1,6 the lowest value thus far observed in pneumonia patients was 2.17 mg %. Data at hand indicate the normal average plasma amino acid uitrogen to be 4.50 mg per 100 cc with the standard deviation ±0.46.

All patients in the present series were treated with sulfapyridine. Unpublished data indicate that in therapeutic doses this drug has no effect upon the plasma amino acid concentration.

Studies are at present under way in this clinic on the plasma amino acid nitrogen concentration in a variety of acute infectious diseases and in a few selected metabolic disorders. Until additional data have been obtained, the physiological significance of hypoaminoacidemia cannot profitably be discussed.

Summary. Observations on the plasma amino acid nitrogen of 6 patients with pneumocoecal pneumonia are presented. In each instance during the acute phase of the disease the patient showed a plasma amino acid concentration significantly below the average normal value. During convalescence there was a gradual rise in the concentration of plasma amino acid nitrogen, with a return to a normal level on complete recovery from the disease. Present data indicate the normal average value to be 4.50 mg per 100 cc.

⁵ Van Slyke, D. D., J. Biol. Chem., 1929, 83, 425.

⁶ Farr, L. E., Am. J. Dis. Child., 1939. 58, 939.

11434

Squid Melanin: A Naturally Occurring Reversibly Oxidizable Pigment.*

FRANK H. J. FIGGE.

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It was reported previously that synthetic melanin resulting from either the enzymatic oxidation of tyrosine or auto-oxidation of dihydroxyphenylalanine was reversibly oxidizable. It was also found that natural melanin was reversibly oxidizable but that the reaction was far more sluggish and barely perceptible. This was thought to be due to the necessarily low concentrations obtainable and the contaminations present in the solutions of natural melanin.

The change in percentage light absorption on reduction of synthetic melanin was very marked. It was thought that these could be duplicated if a source of relatively pure and concentrated natural melanin could be found. Such a source was found in the so-called ink of the ink-sac of the squid (Loligo pealii). The ink is a highly concentrated, relatively pure colloidal solution of melanin. The particles were so small that they were invisible under the ordinary microscope, but visible with dark field illumination.

The ink was collected by removing the squid from the water with a net as carefully as possible to prevent discharge of the ink. The mantle was slit immediately with a pair of long scissors. The ink-sac was then dissected out without much danger of being discharged. Many attempts to anesthetize or immobilize the animal by cooling almost always caused the discharge of most of the ink. The ink was withdrawn with a syringe or expressed into a vial after the sphincter had been removed. The ink collected in this manner was extremely concentrated. After diluting with 300 volumes of water, au ink solution, approximately 1 cc in thickness, would transmit an amount of light that was barely registered by a sensitive photoelectric colorimeter.

For purposes of comparison, a solution of dopa melanin was prepared by auto-oxidation in air. The concentrations of the squid melanin and the dopa melanin solution were adjusted so that they

^{*} Aided by a grant from the American Association for the Advancement of Science.

¹ Figge, F. H. J., PROC. Soc. Exp. BIOL. AND MED., 1939, 41, 127.

both absorbed 90% of the light. Both solutions were then reduced with sodium hydrosulphite and the light absorption values again determined. The reduced dopa melanin absorbed 66% of the light, while the reduced squid melanin absorbed 76% of the light. The melanin solutions were then re-oxidized with potassium ferricyanide. The re-oxidized dopa melanin absorbed 89% of the light, while the re-oxidized squid melanin absorbed 88.5%. This experiment was repeated 22 times with ink from 35 squid. The results were always uniform.

It may be seen from the light absorption values that the reversibility of the oxidation of the natural squid melanin approached that of synthetic dopa melanin. The figures indicate that the squid melanin was contaminated with some substance that absorbed about 10% of the light and which was not reversibly oxidizable. This substance may have been melanin that had aged in the squid ink-sac because even synthetic melanin 6 months old was not reversibly oxidizable.

11435 P

Relationship between "Spreading Factor" and Hyaluronidase.*

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Duran-Reynals¹ demonstrated the presence of an extractable factor in certain tissues and bacteria which enhances the invasiveness of some pathogenic agents. This factor has been extensively studied by Duran-Reynals and by others.^{2, 3} Chain and Duthie⁴ reported that testis extracts containing "spreading factor" decrease the viscosities of synovial fluid and vitreous humor with the liberation of reducing substances. They suggested that "spreading factor"

^{*} This work has been supported in part by a grant from the John and Mary Markle Foundation.

¹ Duran-Reynnls, F., Compt. rend. Soc. biol., 1928, 90, 6; J. Exp. Med., 1929, 50, 327; ibid., 1933, 58, 161; Yale J. Biol. and Med., 1939, 11, 601.

² McClean, D., J. Path. and Bact., 1930, 33, 1045; ibid., 1936, 42, 477.

³ Claude, A., J. Exp. Med., 1937, 66, 353; Claude, A., and Duran-Reynals, F., J. Exp. Med., 1937, 65, 661.

⁴ Chain, E., and Duthie, E. S., Nature, 1939, 144, 977.

is probably identical with the "mucinase" which hydrolyzes the polysaccharide in these fluids.

A mucopolysaccharide designated as hyaluronic acid has been isolated from vitreous humor, unbilical cord,⁵ the mucoid phase of Group A hemolytic streptococci,⁶ synovial fluid,⁷ fowl sarcoma⁸ and the pleural fluid of a patient with a mesothelioma.⁹ Enzymes which hydrolyze hyaluronic acid have been prepared from pneumococci, Group A hemolytic streptococci, Cl. welchii, and splenic tissue.¹⁰ More recently Meyer and Chaffee¹¹ confirmed the observation of Chain and Duthie and demonstrated that testis extracts hydrolyze isolated hyaluronic acid as well as the polysaccharide acid of cornea. The presence of hyaluronidase in high concentration has also been demonstrated in leech extract.¹²

The present study was undertaken to determine more precisely the relationship between "spreading factor" and hyaluronidase. The spreading capacity of the various preparations was tested by the intracutaneous method (using T.1824). The action of hyaluronidase was tested (1) by the hydrolysis of isolated hyaluronic acid and (2) by determining changes in the viscosity of various fluids known to contain hyaluronic acid.†

The presence of hyaluronidase and "spreading factor" was tested in preparations from the following sources: pneumococci (Type I virulent and avirulent strains, Type II avirulent strain). Group A hemolytic streptococci (virulent and avirulent strains in the nucoid and rough phases), Cl. welchii, testis, pigskin, leech extract, commercial hirudine, and certain chemical substances.

Results. All preparations containing hyaluronidase were found to possess spreading properties. In addition hyaluronidase and "spreading factor" were found to possess certain attributes in common. The activity of both was weakened by heating at 65°C for

⁵ Meyer, K., and Palmer, J. W., J. Biol. Chem., 1936. 114, 689.

⁶ Kendall, F. E., Heidelberger, M., and Dawson, M. H., J. Biol. Chem., 1937, 118, 61.

⁷ Meyer, K., Smyth, E. M., and Dawson. M. H., J. Biol. Chem., 1939, 128, 319.

⁸ Kabat, E. A., J. Biol. Chem., 1939, 130, 143.

⁹ Meyer, K., and Chaffee, E., J. Biol. Chem. 1940, 133, 83.

¹⁰ Meyer, K., Hobby. G. L., Chaffee, E., and Dawson, M. H. J. Exp. Med., 1940, 71, 137.

Meyer. K., and Chaffee, E., Proc. Soc. Exp. Biol. and Med., 1940, 43, 487.
 Claude, A., Proc. Soc. Exp. Biol. and Med., 1940, 43, 684. Unpublished data.

[†] The assumption has been made by some investigators that capacity to reduce viscosity constitutes a test for the presence of "mucinase." While it is apparently true that hydrolysis and decrease in viscosity are catalyzed by the same agents, it is not certain that the two reactions are due to the same enzyme.

30 minutes and destroyed at 100°C; both were destroyed or markedly weakened by iodine and, under the experimental conditions, neither was reactivated by sodium sulfite.

The theory that spreading factor and hyaluronidase are identical postulates the existence in skin of either hyaluronic acid or a similar substrate on which hyaluronidase may act. Recently, in this laboratory, a polysaccharide acid which is hydrolyzed by hyaluronidase preparations has been isolated from skin. The nature of this polysaccharide will be described elsewhere.

The evidence so far presented suggests that spreading factor may owe its action to the presence of hyaluronidase. On the other hand, certain observations suggest that the two are not identical.

A number of preparations which possessed marked spreading action did not hydrolyze hyaluronic acid nor did they reduce the viscosity of solutions containing the polysaccharide. Among these were several preparations from different strains of Group A hemolytic streptococci, pigskin, commercial birudine, arsenious oxide and hyaluronic acid.‡

A further possible point of distinction was observed between hyaluronidase and "spreading factor." Antiserum made against hyaluronidase prepared from pneumococci specifically and completely inhibited the activity of the homologous enzyme but did not inhibit the spreading action of the pneumococcal preparations. This difference may be due to the combination of pneumococcal hyaluronidase with the antiserum to form a loose complex which may be inactive in vitro but may dissociate to an active form in vivo. It should be mentioned, however, that the antiserum to the pneumococcal enzyme did not inhibit the action of hyaluronidase prepared from streptococci nor did it affect the spreading action of such preparations.

Summary. Evidence is presented to show that hyaluronidase and "spreading factor" exhibit certain attributes in common. However, there is also considerable evidence that "spreading factor" does not owe its activity solely to the presence of hyaluronidase. It would seem probable that the phenomenon of "spreading" is a complex one and that several factors may be involved in its production. Further work is required to explain the mechanism of "spreading" in terms of known chemical and physico-chemical reactions.

[†] Duran-Reynals¹ has also shown that skin contains spreading factor. The spreading effect produced by simple chemical substances may be due to the release of "spreading factor" locally at the site of injection.

11436 P

The Sympathetic Component of the Sciatic Nerve.

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From the Departments of Anatomy and Pharmacology, New York University
College of Medicine.

Our present knowledge of the course of the preganglionic nerve fibers is based largely on the early experiments of Langley^{1,2} and Bayliss and Bradford³ who used erection of hairs, sweating and vascular changes for recording sympathetic activity. These types of effector activity do not lend themselves readily to quantitative estimation. Moreover sweating and vasomotor changes offer additional difficulties in that they are complex responses. Sweating is influenced by accompanying vascular changes while vasomotor effects in turn are subject to rapid modification by compensatory reflexes.

In the present experiments a direct insight into the kind and magnitude of the sympathetic nerve impulses themselves was obtained by recording the action potentials in the efferent nerves. A direct current amplifier, which Marrazzi⁴ had found, in recording action potentials from other parts of the sympathetic system, to be especially suitable, was utilized to actuate a Matthews oscillograph. This technique provides not only a means of tracing the pathway of preganglionic fibers to an organ or a limb but enables the analysis of an exact point-to-point representation of the sympathetic component of each ventral root in any peripheral nerve.

Method. In a series of experiments on 19 cats, lightly anesthetized with nembutal or sodium amytal the spinal nerve roots from T7 to L7, inclusive, were severed from the cord, the dorsal root and ganglion excised, and the distal cut end of the ventral root stimulated, after insulation from surrounding tissues, by accurately controlled shocks from a thyratron stimulator. The activity resulting in the sciatic nerve was detected by electrodes placed on the main branches of the ipsilateral nerve and connected to the amplifier. The B and C waves thus recorded enabled us to map the exact roots

¹ Langley, J. N., J. Physiol., 1891, 12, 347.

² Langley, J. N., J. Physiol., 1894-95, 17, 296.

³ Bayliss, W. M., and Bradford, J. R., J. Physiol., 1894, 16, 10.

⁴ Marrazzi, A. S., J. Pharm. and Exp. Therap., 1939, 65, 18.

through which sympathetic fibers passed to the lower limb via the sciatic nerve. Since all slowly conducting fibers are not exclusively autonomic, the possibility that some of the B and C waves might have originated elsewhere was considered and excluded by the fact that the intravenous injection of nicotine, which in the doses given acts at autonomic ganglia without affecting postganglionic nerve trunks, was effective in blocking the previously recorded impulses.

Usually 3 or 4 roots were stimulated in each experiment. By separation of the medial and lateral popliteal nerves and recording from each in turn, while repeating the stimulus to the ventral roots, the sympathetic component of each division of the sciatic nerve was determined. Similarly, by recording from the contralateral sciatic, data were obtained on the extent of extraspinal crossed pathways between the sympathetic chains.

Results. Nicotine injected intravenously in doses producing a ganglionic block abolished both the B and C waves appearing after stimulating L1, L2 and L3. Nicotine has not yet been utilized in the experiments in which the other roots have been stimulated. The results show that in the cat the preganglionic outflow to the lower limb via the sciatic nerve emerges constantly from the spinal cord by the ventral roots as high as T11 and as low as L4, and that the roots giving the greatest contribution are T13-L3 (inclusive) of which L1 and L2 invariably gave a response. T11 rarely contributes and T12 and L4 only occasionally (½-½ of the animals).

From the positive roots B and C waves or C waves alone were recorded. The C waves appeared in all cases except in 2 where the animal was in poor condition. In these apparently only the fibers of lower threshold responded giving B waves alone. Similarly, in several experiments, not tabulated because the records were taken immediately after cessation of the circulation, again B waves only were obtained.

Recording from the medial and lateral popliteal divisions of the sciatic nerve separately gave the same result as recording from the combined nerve. No responses from the contralateral sciatic nerve were ever obtained, if care was taken to avoid spread of current. Crossed pathways, between the sympathetic chains, of fibers destined for the opposite sciatic nerve have therefore not been demonstrated.

The results agree with Langley's^{1,2} analysis of outflow of secretory fibers to the hind limb of the cat, made from naked-eye observations of sweating on the foot pads during ventral root stimulation. In dogs Bayliss and Bradford² used the plethysmographic method of

recording changes in volume in the lower limb during spinal root stimulation, and found vasoconstrictor fibers to the lower extremity emerging in T11-L2 (inclusive) and to a lesser extent in L3. Oughterson, Harvey and Richter⁵ indicate by recording the temperature changes in the lower limb of dogs after interrupting the vasomotor pathways by transection of the spinal cord, that vasoconstrictor fibers to the lower extremity may emerge below L6. Derom⁶ would limit the vasomotor fibers to the first 3 lumbar nerves in the dog, on the grounds that section of the rami communicantes of these roots abolishes all vasomotor reflexes in the lower extremity.

The existence of B as well as C waves, amongst the responses that were shown to be autonomic by their disappearance after nicotine, is of considerable interest. The presence of B fibers in the sympathetic outflow to the limb has not hitherto been clearly indicated, though Erlanger speaks of some "inconstant results" in cats and dogs. Such fibers imply the possibility of control over effectors differing in function and distinct from those supplied by fibers of the C group. The number of B and C fibers contributed by each root would then determine the possible extent of its control over the various types of sympathetic response.

11437

A Difference in Effect of Distilled Water and of Isotonic Solutions in Intestine on Pancreatic Secretion.

J. O. CRIDER AND J. EARL THOMAS

From the Department of Physiology, Jefferson Medical College of Philadelphia.

The fact that water in the intestine stimulates the external secretory function of the pancreas was first demonstrated by Damaskin in Pavlov's laboratory^{1, 2} and later by Babkin^{3, 4} and Bylina.⁵ In the

⁵ Oughterson, A. W., Harvey, S. C., and Riehter. H. G., J. Clin. Invest., 1932, 11, 1065.

⁶ Derom, E., Mém. de l'Acad. Roy. de Méd. de Belg., 1938, 25, 1.

⁷ Erlanger, J., in Erlanger, J., and Gasser, H. S., Electrical Signs of Nervous Activity, Philadelphia, University of Pennsylvania Press, 1937, p. 67.

¹ Pavlov, I. P., Die Arbeit der Verdauungsdrüsen, Weisbaden, 1898.

² Pavlov, I. P., The Work of the Digestive Glands, London, 1910, p. 144.

³ Babkin, B. P., Arch. d. Sci. Biol., 1904, 11, No. 3 (Reference from Babkin⁴).
4 Babkin, B. P., Die aussere Sekretion der Verdauungsdrusen, Berlin, 1914.

⁵ Bylina, A. S., Prakt. Arzt, (russ) 1911, No. 44 49 (Reference from Babkin4).

course of an investigation of the effects on pancreatic secretion of various organic buffer mixtures in the intestine (results to be published elsewhere), we found that many neutral, isotonic, watery solutions were ineffective. The reason for this apparent conflict in results provides the material for the present report.

The dogs used in these experiments were provided with gastric and duodenal fistulas fitted with large cannulas ($\frac{5}{8}$ " lumen). The duo-

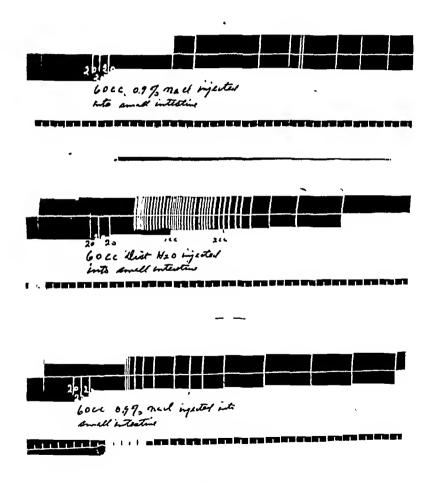


Fig. 1.

Effect of injecting 60 ce of distilled water (middle record) into the intestine compared with the effect of an equal amount of 0.9% NaCl solution (upper and lower records) given 25 min before and 25 min after the water. A drop recorder was used which delivered 17 drops per cc. Time is in 30 sec intervals.

denal fistula was placed opposite the point of entrance of the main pancreatic duct into the duodenum. The pancreatic juice was collected by means of a funnel shaped rubber cup, which was held against the duodenal wall surrounding the papilla by means of a suitable spring device. Further details of the method will be given in the report referred to above.

Water and various solutions were either injected into the upper small intestine in 20, 40, or 60 cc amounts through a tube passed via the duodenal cannula or perfused through the intestinal lumen by means of a pump delivering approximately 15 cc per minute.

Distilled water injected into or perfused through the intestine caused a brief but rapid flow of pancreatic juice. Various isotonic solutions, e.g., 0.9% NaCl and 5% glucose had no such effect, except when given after an injection of distilled water, when a doubtfully positive result was frequently observed (Fig. 1).

Evidently the pancreatic secretagogue action of water is in some way related to hypotonicity and is not exhibited by the water present in isotonic solutions. This fact has an important bearing on the interpretation of experimental data obtained with watery solutions. Instead of concluding, as at present, that a substance in solution is inert if it has the same effect on the pancreas as an equal amount of pure water, we must regard it as an active stimulus if it is effective in isotonic solution.

These observations do not necessarily apply to water or solutions that have passed through the stomach where they may become mixed with HCl or, possibly, other pancreatic secretagogues.

Conclusion. Although pure water in the intestine causes paucreatic secretion, isotonic solutions of inert substances in water do not.

11438

Ovarian Transplantations in the House Mouse.

G. G. ROBERTSON. (Introduced by J. S. Nicholas.)

From Osborn Zaological Laboratory, Yale University, New Haven, Conn.

There have been many attempts to obtain offspring from homoplastic transplants of ovaries in vertebrates. Of these attempts, the most important are those of Magnus¹ on rabbits, Guthrie² on hens, and Castle and Phillips³-⁵ on guinea pigs and rabbits. The results of Magnus and Guthrie are uncertain, however, due to the possibility of the regeneration⁵, of host ovarian tissue. Castle and Phillips grafted ovaries into 141 guinea pigs which differed from the donors in a single genetic character, and, of these, 3 produced young having the genetic characters of the donor animals. The present work deals with the results obtained from ovarian transplantations in the house mouse.

Three groups of experiments were carried out on mice 6-8 weeks old. These are: Group I. A single ovary was transplanted from an animal of one inbred line into an animal of a different inbred line. Group II. A single ovary was transplanted from an animal of an inbred line into an animal differing in a single genetic character but belonging to the same inbred strain as the donor animal. Group III. As in Group II but in which the remaining host ovary was completely removed.

The transplantations were carried out as follows: The ovary of the host animal was exposed by a dorso-lateral incision in the abdominal body wall. A small incision was made in the ovarian capsule as far distant from the Fallopian tube as possible, and the ovary removed. The donor ovary was then pushed through the slit into the host ovarian capsule. No sutures were needed except for closing the incision in the abdominal wall.

Group I. Ovaries from yellow and agouti mice of an inbred line were grafted into 36 female albino mice of 2 different inbred lines.

¹ Magnus, V., Norsk Mag. for Lacgevidensk., 1907, 5R, 5, 1057.

² Guthrie, C. C., J. Exp. Zool., 1907-08, 5, 563.

³ Castle, W. E., and Phillips, J. C., Science, N. S., 1909, 30, 312.

⁴ Castle, W. E., and Phillips, J. C., Carnegie Institution of Washington, 1911, Publ. No. 144.

⁵ Castle, W. E., and Phillips, J. C., Science, N. S., 1913, 38, 783.

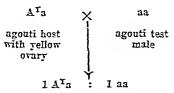
⁶ Davenport, C. B., J. Exp. Zool., 1925, 42, 1.

⁷ Haterius, H. O., Physiol. Zool., 1928, 1, 45.

The host albino females were test mated with albino males and all of the offspring obtained were albino, indicating that none of the ovarian grafts were successful. This is in agreement with the work on tissue transplantation by Loebs and Loeb and Wright on normal tissues of guinea pigs, and by Loeb and King on normal tissues of rats. They found that tissues transplanted between 2 different inbred lines almost invariably degenerate, and few, if any, successful grafts are obtained.

Group II. Ovaries from yellow mice were transplanted into agouti litter mates in 11 cases. The yellow allele is dominant to the recessive agouti allele and there should be some yellow offspring resulting from the mating of the host agouti females to agouti males if the ovarian grafts are successful. However, all of the 30 young obtained in the resulting 5 litters were agouti, showing that the grafts were again unsuccessful.

Group III. Successful ovarian grafts were obtained, however, when one agouti host ovary was completely removed, and, a week to 10 days later, an ovary from a yellow litter mate was grafted into the opposite side of the agouti host. Thus, the agouti host contained one ovary from a yellow litter mate and neither of its own ovaries. Eight such operations have been completed. Of these 8 agouti females 4 have given no litters (indicating either sterility or failure of the graft to take), and four have given a total of 9 yellow and 9 agouti offspring after matings with agouti males. These transplantations were successful, and the young produced are according to genetic expectation. Thus:



In second matings, in which the 4 agouti females containing the successful ovarian grafts were mated with yellow males (for genetic purposes). 3 of the 4 animals have given a total of 8 yellow and 3 agouti offspring. This also is according to genetic expectation, showing that the transplanted ovaries have continued to function. In all of the litter-bearing females in this group the ovarian graft was

⁸ Loeb. L., Physiol. Rev., 1930, 10, 547.

⁹ Loeb. L., and Wright, S., Am. J. Pathol., 1927, 3, 251.

¹⁰ Loeb, L., and King, H. D., Am. Nat., 1935, 69, 5.

successful, and the remainder of the experimental females gave no litters. This shows that there was no regeneration of functional host ovarian tissue. In the animals of Group II, however, the grafts were unsuccessful in all 5 of the females which gave litters. This, according to the results in Group III, is not due to regeneration of host ovarian tissue at the site of the operation, but is probably due to a physiological block between the host ovary of one side and the grafted ovary, since such a high percentage of successful grafts are obtained when the remaining ovary is completely removed. Thus, it seems evident that the presence in the host of one of its own ovaries changes some reaction which is necessary for graft maintenance.

Since the host agouti females of Group III were first mated approximately 10-15 days after the operations, and since 3 have given second litters, it is quite certain that the ovulations in the successfully grafted ovaries were not due simply to the mechanical stimulation of the operation. Additional experiments of this type are being done at the present time, and it is hoped that the high percentage of successful ovarian grafts can be equalled or increased. This type of operation should be of importance as an aid to the solution of problems in developmental genetics of mammals.

11439 P

Effect of Anterior Chordotomy on Essential Hypertension.

OLAN R. HYNDMAN, JULIUS WOLKIN AND W. D. PAUL.

From the Department of Surgery, Neuro Surgical Service, and Department of Internal Medicine, State University of Iowa College of Medicine, Iowa City, Iowa.

Within the past 10 years there have been many reports concerning the surgical treatment of essential hypertension. The methods used both in experimental animals and in man may be classified into 4 different groups: (1) Interrupting sympathetic outflow by cutting anterior nerve roots from T_0 to L_2 , (2) dividing the splanchnic and the sympathetic chain above the diaphragm, (3) dividing the

¹ Braden, S., and Kahn, E. A., Yale J. Biol. Med., 1939, 11, 449.

² Craig, W. M., Surgery, 1938, 4, 502.

³ Ascroft, P. B., Lancet, 1939, 2, 113.

⁴ Page, I. H., and Hener, G. J., Am. J. Med. Sc., 1937, 193, 820.

⁵ Freyberg, R. H., and Peet, M. M., J. Clin. Invest., 1937, 16, 49.

splanchnic nerves and the lumbar sympathetic chain below the diaphragm, and (4) removing the coeliac ganglion.

These various methods, however, have proved to be only partially successful, and this has stimulated us to attempt a new approach to the surgical treatment of essential hypertension. blatt's ingenious experiments on dogs have shown that total sympathectomy of the thorax and abdomen, and even pithing, have had no effects on the type of experimental hypertension he produced. Based on the consideration that various forms of sympathectomy may reduce the blood pressure of patients with essential hypertension, but that these results were often disappointing, it was at first the intention of one of us (O.H.) to effect a more complete sympathectomy by partially sectioning the autonomic tracts in the cord. We observed, also, that when routine anterior chordotomy was performed for reasons other than hypertension, the operation was usually followed by a prolonged lowering of the blood pressure. Later it was found that, as far as the arterial pressure was concerned, a maximum result could be attained by beginning the section one to two mm anterior to the dentate ligament and carrying it to the anterior median fissure, thus interrupting the anterior and anterolateral columns of the cord. Bilateral sections are made because unilateral sections were found to have little effect on the arterial pressure. It has been found that the optimal location for the section is at the 8th cervical segment.

In women with essential hypertension, chordotomy has been almost uniformly followed by a reduction of blood pressure to the normal figure for 4 to 6 months, after which it tends to return to a higher level, but only occasionally to the preoperative level. Moreover, this operation has not necessarily produced a disturbance in sweating. Most of the subjects on whom this operation has been performed have had advanced hypertension, with subjective symptoms, and all of them have been greatly benefited. Our results in the few male patients so treated have not been as successful as the females. The few male patients whom we have subjected to this operation had more advanced changes in the vascular tree than the females so that the difference in sex was probably not the determining factor.

The authors feel that a dual mechanism (central neurogenic, and

peripheral) is probably involved in essential hypertension, and that chordotomy possibly eliminates the central neurogenic factor. Work is being carried out with the idea of proving or disproving this

⁶ Allen, E. V., and Adson, A. W., Am. Heart J., 1937, 14, 415.

⁷ Crile, G., Ann. Surg., 1938, 107, 909.

⁸ Goldblatt, H., Surgery, 1938, 4, 483,

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	Reactions with type-specific sera				
Pneumococcal strain	Maximal capsular swelling and massive agglu- tination	Slightly sub- maximal swell- ing and moderate to massive agglu- tination	Little or no capsular swelling and moderate agglutination		
Hoge*	29	10	20		
Weingart	29	20, 31	_		
Thorpe	29	10, 20			
Hinman	24	7	20		
Brown	24	7	20		
Walker	29	10, 20	_		

^{*} Englewood Hospital Laboratory, Chicago, kindly furnished the specimen from which this strain was obtained.

ing strains. From these the last 5 listed in Table I were recovered. There is reason for believing that such strains were present in 2 other specimens but escaped isolation.

To ascertain that a mixture of types was not responsible for the multiple-type reactions, each of the 6 strains was plated from young blood-broth cultures. From each plate well-segregated colonies were picked, each colony to a fresh tube of blood broth. This procedure was repeated not less than four times with each strain. No change in the reactions of any of the strains was produced by this treatment.

Table II shows the reactions of 4 strains with the sera of 3 principal producers of typing sera. For purposes of comparison and control one strain each of types 10, 20, 29, and 31 also was tested. These were obtained from one of the producers whose sera were included in the study (Manufacturer C). Young cultures in Felton broth, formalinized (1.0% commercial formalin) to inhibit autolysis, or saline suspensions prepared therefrom, were employed in the tests. The saline suspensions were prepared by centrifugalizing the cultures and resuspending the sediment in formalinized (1.0%) physiological saline, the final turbidity approximating that of tube number 8 of a Macfarland nephelometer. One 2 mm loopful of bacterial suspension, one 2 mm loopful of Loeffler's methylene blue and one 4 mm loopful of serum were mixed in each preparation examined under the microscope.

It is apparent from the second table that although the sera from the 3 sources differ somewhat in the scope and degree of their cross-reactions they severally substantiate the fact of multiple-type antigenicity in the strains tested. Even the control strains, types 10 and 20, do not appear to be perfectly specific. However, the reactions of

hypothesis, and, if it should be correct, then eliminating the central factor would leave only the peripheral (hormone?) mechanism which may become more amenable to drug therapy.

Some physiological effects of anterior chordotomy have been discussed. The result of chordotomy on motor function in some 90 cases will be published in the near future.

11440 P

Occurrence of Strains of Pneumocoeci Which React With More Than One Type-Specific Antipneumocoecal Serum.

GEORGE F. FORSTER AND HOWARD J. SHAUGHNESSY.

From the Division of Laboratories, Illinois Department of Public Health,

Chicago, Ill.

Pneumococci are commonly classed among the best examples of bacterial type-specificity. While cross-reactions frequently occur involving types 3 and 8 and less frequently other types, they have usually been considered from the standpoint of the cross-reacting antibodies that sometimes develop during immunization rather than from the standpoint of the antigenic type-multiplicity which causes them.^{1, 2}

Several strains of pneumococci that conspicuously violate the prevailing conception of type-specificity have been isolated recently in this laboratory. Neufeld tests with sera of 3 different manufacturers show that each of these strains reacts with at least 3 type-specific sera.

Table I summarizes the reactions of 6 such strains. It will be noted that each strain shows somewhat greater capsular swelling with one serum (either type 29 or type 24) than with others. However, reaction is only slightly less with a second, and in some strains with a third, serum.

With the exception of the Hoge strain all 6 were isolated from 488 routine specimens submitted to this laboratory for typing between February 16 and April 3, 1940. Fifty-nine of these specimens were reported as containing more than one type of pneumococci, and 18 of them examined for the possible presence of multiple-react-

⁹ Hyndman, O., and Van Epps, C., Arch. Surg., 1939, 38, 1036.

¹ Lyall, H. W., and Odell, H. R., Am. J. Hyg., 1939, 29 (Sect. B), 103.

² Noble, A., and Cameron, B. C., J. Lab. and Clin. Med., 1939, 24, 1.

this group of cultures with the 3 sets of sera are close enough to strict specificity to furnish further evidence of the multiple-antigenicity of the test-strains. The somewhat discrepant results obtained with the sera of the 3 manufacturers may have either of two explanations: (1) cross-reacting antibodies may have been more completely removed from one set of sera (by absorption) than from another, or (2) more strictly specific cultures may have been used as antigens in the production of one set of sera than in another.

While measurement by loops, as in the Neufeld test, is not a perfectly quantitative method, the results here cited have been obtained so repeatedly and consistently that they leave no room for doubt as to their reproducibility.

From the figures cited above it seems likely that the occurrence of these broadly non-specific strains is fairly frequent. Their significance for the prevailing conception of type-specificity is obvious. Recognition of their existence creates a number of problems in the diagnosis and treatment of pneumonia, some of which are being studied further.

						Type-Spe	l'ype-Specific Sera					
		Manufacturer A	turer A			Manufa	Manufacturer B			Manufa	Manufacturer C	
Cultures	10	20	65	31	10	30	es:	E E	10	30	29	31
Multiple-reacting str Hoge Weingart Walker Thorpe Control strains Type 10	# + + + +	++++ + ++++ + ++++ ++	++++ + ++++ + ++++ +	S: + + + + + + + + + +	+1+++311	+++ \ + + + + + + + + + + + + + + + + +	++++ + ++++ + ++++ +		+ + + + + + + + + + + + + + + + + + +	++++++++++++++++++++++++++++++++++++++	++++ + ++++ +	+ + + + +
+++ maxin = +++	= maximal capsular swelling.	r swelling										

++= slightly swelling.
+= definite swelling.
== doubtful swelling, moderate to heavy agglutination.
Ag. == moderate to heavy agglutination, no swelling.
Sl.Ag. = slight agglutination, no swelling.

- = no agglutination, no swellin

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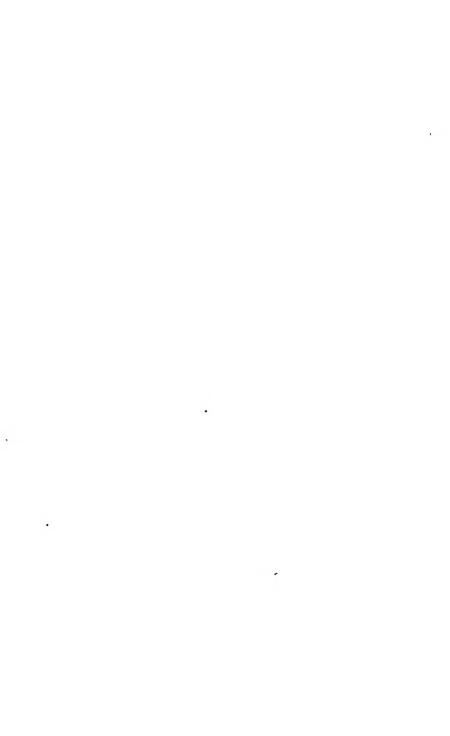
Isolation of an Unpigmented Skin Reactive Constituent from Extracts of Ragweed Pollen by Electrophoresis.*

H. A. Abramson, D. H. Moore, H. Gettner, J. Gagarin and L. Jennings.

Prom the College of Physicians and Surgeons, Columbia University, the Mount Sinai Hospital, New York City, and the Biological Laboratorics, Cold Spring Harbor, Long Island,

In 1938 experiments were undertaken, using a conventional moving boundary technique, to separate the fractions and to determine the electrical charge of the constituents of giant ragweed pollen extracts. At that time it was observed that the pigments in dialyzed ragweed extracts at pH 7.0 were negatively charged. The results during that summer and the succeeding winter, however, were contradictory, because the relation of the pigments to the biologically active constituents was uncertain.

^{*}This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.



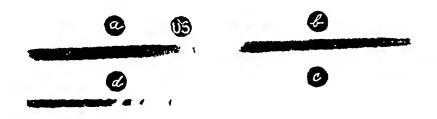


Fig. 2.

To conserve space, these Schlierung or band patterns of the constituents of extracts of ragueed pollen have been rotated 90° with the top of the electrophoresis cell on the left hand side. The US fraction is indicated by the broad band in Fig. 2a. For description of sections b, c, d, see text.

vancing column of pigmented constituents and shows many bands with electrical mobilities closely related to one another but with very low concentrations of each. These bands are essentially similar to those illustrated in Fig. 1b.

It has been previously observed by one of us that the skin reactive constituents of giant ragweed extract may be introduced by an electrical field into the skin by either the negative or the positive pole. Indeed, it was surprising to observe that the positive pole was more efficient than the negative pole with unfractionated dialyzed solutions at pH 7.0. The present studies show that there was no positively charged component observable by the method in the six dialyzed extracts thus far studied. The absence of a positively charged skin reactive constituent is evidence in favor of the point of view of Abramson and Gorin* that diffusion forces primarily account for the movement of the skin reactive constituent into the skin during the passage of the current.

Further experimental evidence that the positive pole may transport a negatively charged skin reactive constituent was obtained in the following way. A sample of the unpigmented active fraction was dialyzed for one hour against distilled water to remove phosphate buffer. It was then brought by addition of sodium hydroxide to pH 7.0. The nitrogen content of this solution was 0.25 mg/cm³. Using this dialyzed negatively charged constituent sufficient ragweed was introduced by electrophoresis from the positive pole for 3 minutes to produce a severe skin reaction in an individual markedly skin-sensitive to ragweed pollen.

Some idea of the skin reactivity of the unpigmented fraction in

⁴ Abramson, H. A., and Gorin, M. H., J. Physical Chem., 1939, 43, 3; Abramson, H. A., and Gorin, M. H., Chem. Prod., 1940, 3, 37.

Using the Tiselius1 cell for the study of the moving boundaries of protein solutions and the Philpot-Svensson" technic to analyze the quantity and nature of the constituents, further progress may now be reported. Figures 1a and 1 b are illustrations of the type of curves given by the major constituent. This major constituent is negatively charged, slow moving, unpigmented, and highly skin reactive in persons sensitive to ragweed. A minor constituent, about 1/5 that of the unpigmented major constituent, appearing in the nigment moves approximately 10 times as fast as the unnigmented major one in Fig. 1a. The pigments apparently did not all migrate with the minor boundary but also moved towards the positive pole. Fig. 1b, which also illustrates as a major constituent an unpigmented component has, in addition, several minor constituents migrating towards the positive pole. The electric mobility of the unpigmented skin reactive constituent is $0.05 \,\mu/\text{sec}$ at 1.5°C . It is of interest to note that the electrical mobility of quartz particles in similar ragweed solutions studied by Abramson, Sookne and Moyer^a approximately agrees with this value when temperature corrections for viscosity are made.

In Fig. 2, the section of the electrophoresis cell labelled (a) shows a Schlierung pattern of the boundary between the unpigmented slow moving active constituent and the buffer; section (b) shows a lightly pigmented section; section (c) was so highly pigmented that practically no light came through; and section (d) contained the ad-



Fig. 1a.

The white area labelled US is the Philpot-Svensson curve of the unpigmented, skin reactive, slow moving constituent. The minor peak is a faster moving constituent in the pigment.



Fig. 1b.

This curve was obtained from another sample of ragweed and illustrates again the US fraction as well as four minor constituents migrating in or

with the pigment.

¹ Tiselius, A., Trans. Farad. Soc., 1937, 33, 524.

² Svensson, H., Koll. Z., 1939, 87, 190.

³ Abramson, H. A., Sookne, A., and Moyer, L. S., J. Allergy, 1939, 10, 317.

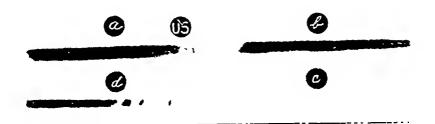


Fig. 2.

To conserve space, these Schlierung or band patterns of the constituents of extracts of ragweed pollen have been rotated 90° with the top of the electrophoresis cell on the left hand side. The US fraction is indicated by the broad band in Fig. 2a. For description of sections b. c, d. see text.

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relation to its nitrogen content is given by the fact that a 1:1000 dilution of a solution containing 0.3 mg of nitrogen/cm² still retained skin reactivity in a ragweed-sensitive case. That is, a solution containing 0.0003 mg of nitrogen/cm³ scratched into the skin by the usual technic, gave a positive test.

The skin reactivity of the pigmented fractions has not as yet been investigated nor have undialyzed solutions been examined electrophoretically.

11442

Absorption Rates and Biologic Effects of Pellets of α-Estradiol and α-Estradiol Benzoate in Women.

ROBERT I. WALTER, SAMUEL H. GEIST AND UDALL J. SALMON.
From the Gynecological Service of Dr. S. H. Geist, Mt. Sinai Hospital, New York:

In a preliminary communication, we have reported the subcutaneous implantation of crystals of α-estradiol benzoate in a group of 10 menopausal patients who had well-defined morphologic signs and symptoms of estrogen deficiency.¹ It was shown that, by this method of administering estrogens, it was possible to achieve a strikingly more prolonged effect than is obtained with comparable amounts of the hormone, administered parenterally, in solution in oil. It was subsequently demonstrated that a correspondingly prolonged inhibition of the hyperactive hypophysis occurred following the estrogen implantation.²

Since Deanesley and Parkes^{3, 4} have shown that prolonged estrogenic effects resulted from the subcutaneous implantation of pellets of estrogens in rats, we thought it desirable to study in women the duration of the physiologic and therapeutic effects of pellets as compared with crystals of the same estrogenic substance. Accordingly, 46 patients were implanted with pellets and 55 with crystals of either α -estradiol or σ -estradiol benzoate. During a period of observation of approximately one year, it was noted that more prolonged physiologic and therapeutic effects re-

¹ Salmon, U. J., Walter, R. I., and Geist, S. H., Science, 1939, 90, 162.

² Salmon, U. J., Geist, S. H., and Walter, R. I., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 424.

³ Deanesley, R., and Parkes, A. S., Proc. Roy. Soc. B., 1937, 124, 279.

⁴ Deanesley, R., and Parkes, A. S., Lancet, 1938, 2, 606.

sulted from the implanted crystals than from pellets of similar weight and chemical constitution. We, therefore, felt it important to determine the rate of absorption of the hormone by removing and reweighing the implanted pellets at varying intervals after the implantation. At the same time, the duration of biologic effects of the implanted hormone, as manifested by morphologic changes in the endometrium and vaginal mucosa, were studied by means of repeated vaginal smears and vaginal and endometrial biopsies. Here we wish to report the results of our studies on the absorption rate and duration of biologic effects of the implanted estrogen pellets.

Material and Procedure. From the 46 cases implanted with pellets, 14 patients (9 natural menopause, 4 surgical castrates and 1 X-ray castrate) were selected for excision. The duration of the menopause, in this group, varied from 2 months to 7 years. All of the patients had either clinical or morphologic evidence of estrogen deficiency, or both, prior to the implantation.

Round, flat pellets of α -estradiol and α -estradiol benzoate.* sterilized by autoclaving (265°F, at 15 lb pressure, for 30 minutes), varying in weight from 15 to 25 mg each, were implanted, subcutaneously, in the outer aspect of the thigh. Nine patients were implanted with a single pellet: 4 with 2 pellets: and one with 3 pellets. In 8 cases, the pellets were of α -estradiol and in 6, α -estradiol benzoate. The skin was prepared with alcohol and iodine and anesthetized with 1% novocaine. The pellets were implanted, approximately 3/4 of an inch below the surface into the subcutaneous fat, through a skin incision approximately 1/2 inch in length. The implantation sites were excised at varying intervals after the implantation and the pellets were weighed after drying in a desiccator.

Results. Absorption Rates of a-Estradiol Pellets. The a-estradiol pellets were excised at periods of time varying from 130 to 245 days following the implantation. Each pellet was found to be closely enveloped by a fibrous capsule. Microscopic study of the surrounding tissues revealed a typical non-specific foreign body reaction. (The histologic details of the tissue reaction to the pellets are described elsewhere.⁵) The results of this study are presented in Table I.

The rate of absorption, expressed in terms of average percent

^{*} For the a-estradiol and a-estradiol benzoate pellets used in this investigation, we are indebted to Dr. Erwin Schwenk, Schering Corporation, Bloomfield, N. J., and to Mr. Robert C. Mautner, Ciba Pharmaceutical Products, Summit, N. J.

⁵ Geist, S. H., Walter, R. I., and Salmon, U. J., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 712.

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⁴ Deanesley, R., and Parkes, A. S., Lancet, 1938, 2, 606.

weight loss per 30 days, varied from 1.9 to 8, with an average for the series of 4.85%. This represents, in terms of rat units, average daily absorption rates varying from 191 to 1460 R.U., with an average for the series of 473 R.U. per day. Deanesley and Parkes, in their study of α -estradiol pellets in rats, reported 6% to 9% average absorption per month.

Absorption Rates of a-Estradiol Bensoate Pellets. The a-estradiol benzoate pellets were excised at intervals varying from 87 to 207 days after the implantation. In this series, also, a fibrous capsule was found surrounding each pellet. The rate of absorption in terms of average percent weight loss per 30 days, varied from 0.41 to 4.7. The average rate of absorption (per 30 days), of this series, was 1.72%. This represents, in terms of rat units, variations from 41 to 238 R.U. per day, with an average for the series of 113 R.U. per day.

Biologic Effects of a-Estradiol Pellets. All cases showed characteristic morphologic evidence of estrogenic effect, as indicated by proliferative response in the vaginal mucous membrane (smears and biopsies) and/or the endometrium, within 2 weeks after the implantation. Details of the effect of implanted estrogens upon the mucous membranes of the genital tract are being reported elsewhere.

At the time of removal of the implanted α -estradiol pellets, there was evidence (in all but 3 cases) of morphologic regression to the pre-implantation status, indicating varying degrees of estrogen deficiency. Apparently, at the time of excision, so little of the hormone was being absorbed that no estrogenic effect was demonstrable in the uterine or vaginal nucosa. In all of these cases the pellets had been retained for 180 days or more.

The 3 cases in this series that revealed morphologic evidence of continued estrogen activity, at the time of excision, had all been implanted for shorter periods of time (23, 130 and 160 days).

Biologic Effects of α -Estradiol Benzoate Pellets. In the α -estradiol benzoate series, morphologic studies, at the time of excision (87 to 207 days post-implantation), revealed, in all cases, regression to the pre-implantation status, indicating the cessation of estrogen activity.

Clinical Effects of a-estradiol Pellets. One patient (Case No. 6) experienced no relief of symptoms; 4 were relieved for periods varying from 101 to 190 days; 2 (Case No. 2 and Case No. 4) were still symptom-free at the time of excision (160 and 130 days post-implantation).

Clinical Effects of α -Estradiol Benzoate Pellets. Two patients (Case No. 9 and Case No. 13) experienced no relief of symptoms following the implantation: 5 were relieved for periods varying

TABLE I.

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Clinical status at excision		No recurrence 75%	100% recurrence 75%	70 improvement 100% recurrence 100% "1 100% "1 No "1	No improvement 100% recurrence	t averige of a-estradiol beuzoate series = 113 R.U. per day. n = matiral menopause. s = surgical menopause. v = x-ray menopause. a-ved = a-estradiol. a-ved = a-estradiol.
Maximum duration of thera- peutie	138 days 160 plus	130 plus 134 days	101 190 ''	60 " 60 " 62 "	fo days	ics ~ 113
Avg daily No. of rat units absorbed, R.U.	308	112	191	125 119 238 82	13844	nzoate ser rate,
Absorp- tion per 30 days, i	0.00) # 10 m	1.9 4.6 <u>%</u>	1:3 1:3 0.81 0.81	17.7	atradiol be nopause, nopause, nause, iol,
Amount absorbed, mg	4.0 a) C & +	. e. i.c. c	34944 84044	3.6	1 averinge of a-estradiol Deuzos 1 = natural menopause. 2 = surgical menopause. 3 = v-ray menopause. 4 - x-ray menopause. 5 - cestradiol. 6 - cestradiol.
Orning of implantation, days	193 160	130 180 130	161 61 151 61 151 61	207 101 116 88	156	n n n n n n n n n n n n n n n n n n n
Wt on removal, mg	18.6	12.0 16.4	21.1 0.6 1.0 4	20.5 20.5 20.5 20.5 20.5 20.5 20.5 20.5	46.4	ent times. per 30 days. = 1,72% per 30 days. U. ner day.
Initial wt, mg	13:12:2	ដូនជ	955	ลิลลิล	20.	imes. 30 days. 72% per 21 day.
Estrogen	164. 11	2 2 2)))) ((= =	different t 4.85% per series — 1.5 473 R.U. ne
Post- menopause no	17 98 1 8	. ၁ % E	00 to 48	5T 95 67 6	÷ c1	series — benzonte series —
n Menopause) c = s	: e = =			× 00	† 2 ' 17 ' This patient was implanted at 2 different times. This patient was implanted at 2 different times. Average of a cestradiol benzoate series = 1.72% per 9 Average of a estradiol series = 473 R.H. ner day.
Age	42 42 43 44	444	84 E	51 52	38 pellets.	This patie Average of Average of Average of
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Clinical Effects of α -estradiol Pellets. One patient (Case No. 6) experienced no relief of symptoms; 4 were relieved for periods varying from 101 to 190 days; 2 (Case No. 2 and Case No. 4) were still symptom-free at the time of excision (160 and 130 days post-implantation).

Clinical Effects of a-Estradiol Benzoate Pellets. Two patients (Case No. 9 and Case No. 13) experienced no relief of symptoms following the implantation: 5 were relieved for periods varying

from 45 to 75 days; one (Case No. 12) was symptom-free at the time of excision, which was 88 days after the implantation. It is apparent from this study that pellets of α -estradiol have a more prolonged therapeutic and biologic effect than pellets of α -estradiol benzoate.

It is important to note that despite the presence of sizeable pellets (weighing 11 to 46.4 mg) in the subcutaneous tissue, the majority of the patients, at the time of excision, exhibited elinical as well as morphologic evidence of estrogen deficiency. The fact has already been mentioned that the pellets were found to be completely enveloped by a tight, fibrous eapsule.⁵ Apparently the eapsule acts as a barrier, progressively retarding absorption of the hormone and reducing it finally to a level at which no demonstrable estrogen effect is exerted, either clinically or morphologically.

The question may be raised as to whether the hormone may not be inactivated by its prolonged contact with the subcutaneous tissues. Such a qualitative change in the pellets has been eonsidered and apparently ruled out by demonstrating that the excised pellets, when reimplanted in rats, produced characteristic estrogen effects.

It is evident from this study that, in spite of the striking initial morphologie and therapeutic effects produced by the implanted estrogen pellets, there is a serious objection to this method of administering estrogens clinically, since the therapeutic effect is relatively short-lived and the patients, thereafter, retain sizeable pellets without deriving any therapeutic benefit from them.

Summary and Conclusions. A comparative study of the absorption rates and duration of biologic effectiveness of implanted pellets of a-estradiol and a-estradiol benzoate was made in a series of 14 eases. This study revealed that the average percent absorption rate, per 30 days, of α -estradiol pellets was 4.85% (= 473 rat units per day), as compared to 1.72% (= 113 rat units per day) for pellets of a-estradiol benzoate. The duration of biologie and therapeutic effects was definitely longer in the α -estradiol series. eluded, on the basis of these studies, that the fibrous capsule which forms about the pellets progressively decreases the rate of absorption of the hormone, so little being absorbed finally that no demonstrable morphologic or therapeutic effect is produced. Furthermore, because of the fact that absorption of the hormone in effective amounts ceases when only a relatively small amount of the pellet has been absorbed, it is concluded that the implantation of pellets (weighing 15 to 25 mg) of α -estradiol and α -estradiol benzoate is not a satisfactory method of administering estrogens clinically.

11443 P

Inhibitory Action of Testosterone Propionate on the Human Ovary.

S. H. Geist, J. A. Gaines and U. J. Salmon.

From The Mount Sinai Hospital, New York City.

In previous communications,^{1, 2, 3} it has been shown that, by the administration of adequate amounts of testosterone propionate to cyclical women, menstruation can be suppressed and the secretory phase of the endometrium abolished, resulting in hypoplasia or atrophy of the endometrium. These effects of testosterone propionate were interpreted as indicating inhibition of ovulation (probably mediated through the hypophysis) with consequent suppression of estrogen and progesterone formation.

In animals, some workers have reported that synthetic androgens have a stimulating action upon the ovaries of rats⁴⁻⁸ and mice.⁹ Others have reported inhibition of ovulation in rabbits¹⁰ and monkeys¹¹ and ovarian atrophy in rats.^{12, 13}

In the study reported here, an attempt was made to determine what effect testosterone propionate has upon the ovaries of cyclical women and to correlate the ovarian response with the endometrial and vaginal changes.

Two regularly cyclical women, requiring exploratory laparotomy, were selected for this study. Endometrial biopsies were taken

¹ Gaines, J. A., Salmon, U. J., and Geist, S. H., Proc. Soc. Exp. Biol. and Med., 1938, 38, 779.

² Geist, S. H., Salmon, U. J., and Gaines, J. A., Endocrinology, 1938, 23, 784.

³ Salmon, U. J., Geist, S. H., and Walter, R. I., Am. J. Obs. and Gyn., 1939, 38, 264.

⁴ Korenchevsky, V., Dennison, M., and Hall, K., Biochem. J., 1937, 31, 780.

⁵ Wolfe, J. M., and Hamilton, J. B., Proc. Soc. Exp. Biol. AND Med., 1937, 37, 189.

⁶ McKeown, T., and Zuckerman, S., Proc. Roy. Soc., London, s.B., 1937, 124, 362.

⁷ Salmon, U. J., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 352.

⁸ Nathanson, I. T., Franseen, C. C., and Sweeney, A. R., Jr., PROC. Soc. EXP. BIOL. AND MED., 1938, 39, 384.

⁹ Starkey, W. F., and Leathem, J. H., Proc. Soc. Exp. Biol. and Med., 1938, 39, 218.

¹⁰ Cotte, G., Martin, J. F., and Mankiewicz, E., Gynecologic, 1937, 36, 561.

¹¹ Zuckerman, S., Lancet, 1937, 2, 676.

¹² McEuen, C. S., Selye, H., and Collip, J. B., PROC. Soc. EXP. BIOL. AND MED., 1937, 36, 390.

¹³ Mazer, M., and Mazer, C., Endocrinology, 1939, 24, 175.

before and during the period of testosterone propionate administration, at intervals of 1 to 2 weeks. Vaginal smears were taken 3 times weekly. Testosterone propionate was administered for 31 and 15 days, respectively, the total amounts being 925 and 1,225 mg. The histologic findings of the ovaries were correlated with the endometrium and vaginal smears. A resumé of the protocols follows:

Case I. Age 29. Gravida 1. Para O. Menses lasting 5 days occurred at regular intervals of 4 weeks. A preliminary endometrial biopsy, taken during the menstrual period, revealed the presence of secretory changes. It was assumed, therefore, that an ovulatory cycle had just been completed. Preliminary vaginal smears were of the normal physiologic type. Testosterone propionate* injections were started 3 days pre-menstrually and continued, at 1 to 3 day intervals, until 925 mg had been administered over a period of 31 days. Menstruation failed to take place by the 34th day of the cycle, at which time operation was performed. At this time, the endometrium was reduced to a state of hypoplasia, while the vaginal smears revealed typical estrogen deficiency characteristics. The ovaries showed no gross evidence of a mature follicle or recent corpus luteum. Two longitudinal sections were made through the entire width of each ovary down to the hilus and the central wedges removed for histologic study. Microscopic examination revealed the presence of small, collapsed or cystic corpora lutea of previous cycles, but no maturing follicles or current corpus luteum.

Case 11. Age 47. Grazida 2. Para 2. Menses had occurred quite regularly at 26 to 28 day intervals, lasting for 5 to 6 days. The patient entered the hospital because of lower abdominal pain related to utcrine fibromyomata. An endometrial biopsy, taken pre-menstrually, revealed a typical secretory phase. Preliminary vaginal smears revealed a normal estrogen effect. During the first 16 days of the next cycle, 1,225 mg of testosterone propionate was administered. A supravaginal hysterectomy and bilateral salpingo-oophorectomy was performed on the 17th day. The endometrium showed moderate proliferation and a complete absence of secretory phenomena. The vaginal smear, at this time showed early signs of regression. Microscopic sections of the ovaries failed to reveal any signs of a recent corpus luteum or maturing follicle.

Summary and Conclusions. Two women with regular menstrual

^{*} For the testosterone propionate used in this investigation, we are indebted to Dr. Erwin Schwenk, Schering Corporation, Bloomfield, N. J. (Oreton), and to Mr. Robert C. Mautner, Ciba Pharmaceutical Products, Summit, N. J. (Perandren).

cycles were injected with testosterone propionate (925 and 1,225 mg), in order to determine whether ovulation could be inhibited. In one patient, the ovaries, examined on the 34th day of the cycle, showed no evidence of a recent corpus luteum or mature graafian follicle. In the second patient, examination of the ovaries, on the 17th day of the cycle, did not reveal any evidence of ovulation. In the latter case, while ovulation might have occurred after the 17th day, it was deemed unlikely in an individual with a regular 26 to 28 day cycle.

It appears from this study that testosterone propionate, if administered in adequate amounts to the cyclical human female, can inhibit full follicle maturation, ovulation and corpus luteum formation, associated with regressive changes in the endometrium and vaginal mucosa. The question arises as to whether the testosterone propionate acts directly upon the follicular apparatus or indirectly through inhibition of the gonadotropic activity of the pituitary. In view of the fact that testosterone has been shown to suppress the gonadotropic activity of the hypophysis in post-menopausal women^{14, 15} and rats, ^{16–18} it is logical to conclude that the inhibitory effect of testosterone propionate upon the human ovary is mediated through the pituitary.

11444

Effectiveness of Sulfanilamide upon Anaerobic Hemolytic Streptococci.

E. H. Spaulding and Amedeo Bondi, Jr. (Introduced by J. A. Kolmer.)

From the Department of Bacteriology and Immunology, Temple University School of Medicine, Philadelphia, Pa.

Previous work in this laboratory¹ has indicated that on primary isolation a significant proportion of hemolytic streptococci are incapable of developing upon the surface of aerobic, infusion blood-

¹⁴ Salmon, U. J., PROC. Soc. Exp. Biol. and Med., 1937, 37, 488.

¹⁵ Nathanson, I. T., and Towne, L. E., Endocrinology, 1939, 25, 754.

¹⁶ Nelson, W. O., and Gallagher, T. F., Anat. Rec., 1935, 64, 129.

¹⁷ Wolfe, J. M., and Hamilton, J. B., Endocrinology, 1937, 21, 603.

¹⁸ Allanson, M., Proc. Roy. Soc., London, s.B., 1937, 125, 196.

¹ Spaulding, E. H., and Goode, W., J. Lab. and Clin. Med., 1939, 25, 305.

agar plates. Although most of these "anaërobic" isolations become quickly adapted to aërobic cultivation (temporarily anaërobic), a small percentage persist as obligate anaërobes. As a result of the clinical observation that several patients infected with obligately anaërobic hemolytic streptococci (Group A) responded poorly to sulfonamide-therapy, two such strains were selected for experimental study. The results are being reported because of the increasing interest in the relationship between anaërobiosis and sulfanilamide activity.²⁻⁵

Both strains reacted with Group A antiserum, and fermented trehalose but not sorbitol. Strain S, originating from a case of bronchiectasis, was unable to grow on aërobic blood-agar for 18 months. The other (1097) was isolated from a hand lesion. After 14 months it began to develop aërobically. Strain S was characterized by mucoid colonies on blood agar, whereas those of 1097 were of the smooth type. On benzidine blood agar⁷ both cultures gave rise to black colonies within two hours after removal from anaërobic jar.

Experimental infection in mice was produced with considerable difficulty since only after prolonged passage did death regularly follow the injection of several million cells. It should be noted that streptococcal strains of low virulence do not usually show marked response to sulfanilamide in mice. presumably because of the large number of organisms in the inoculum. Infection was produced intraäbdominally and the drug administered subcutaneously in 10 mg doses per 25 g body weight. The results are summarized in Table I.

It will be noted that neither treatment-schedule was intensive. Nevertheless, it seems evident that infection induced by one strain (S) was definitely refractory to treatment, whereas infection with the other strain (1097) was moderately susceptible to sulfanilamide therapy. Comparative experiments concerning the effect of fresh preparation of sulfanilamide and samples of the drug oxidized by exposure to air for 30 days yielded similar results.

² Fox, C. L., German, B., and Janeway, C. A., Proc. Soc. Exp. Biol. and Med., 1939, 40, 184.

³ Warren, J., Street, J. A., and Stokinger, H. E., Proc. Soc. Exp. Biol. and Med., 1939, 40, 208.

⁴ Shinn, L. E., Main, E. R., and Mellon, R. R., Proc. Soc. Exp. Biol. and Med., 1939, 40, 640.

⁵ Broh-Kalın, R. H., Science, 1939, 90, 543.

⁶ Marshall, E. K., Jr., Physiol. Rev., 1939, 19, 240.

⁷ MacLeod, C. M., PROC. Soc. EXP. BIOL. AND MED., 1939, 41, 215.

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TABLE I.	
Sulfanilamide	77777
υf	I
Effect	
Therapeutic	

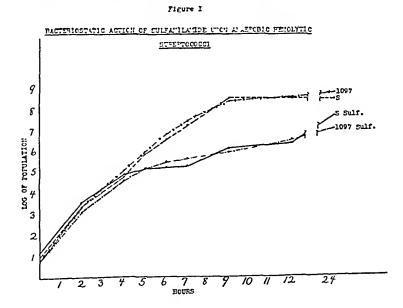
Autonsy	entures Aëro. Anaëro.	+	++	++	++	++
otococci,	Survived			•	x	э 15
Finisms ted Lethal Death of mice in days	7-10 11-25			es -		3 1
Death of mice in days	3 4-6	7	01	5	¢1	61 61
	34 9 17 3	9 8 18	25 5	11		L 4.
	Drug Treated* Controls	Treated Controls	Treated* Controls	Treated* Controls	Treated! Controls	Treated* Controls
ms Lethal	5-10 5-10	10 10	5.10 5.10	$\frac{5.10}{5.10}$	5.10 5.10	101
No. of organisms f injected La (millions)	11 11 11 11 11 11 11 11 11 11 11 11 11	80 80	16 16	e, e,	88 88	9
No. of mice	19 S	18	⊜ro (SE :		; ·
Strain	ಜ್ಞ ಜ	cos o	. s. 501	1007	1097	Trentment:

*20 mg/25 g hody wt daily for 6 days; 10 mg for 5 days (10 mg doses). 10 mg/25 g hody wt daily for 12 days (10 mg doses). 10ccasional minute colony; nërobie subculture negative.

The *in vitro* experiments of Fox, *et al.*,² and of Warren, *et al.*,³ suggest that sulfanilamide would be ineffectual against obligately anaërobic strains. The *in vivo* results with strain 1097, however, indicate that anaërobiosis *per se* may not be an important factor in determining susceptibility of hemolytic streptococci to sulfanilamide-therapy.

In vitro bacteriostasis. Since there appeared to exist unequal responses to sulfanilamide between the 2 strains in mice, it was considered possible that in vitro tests might bring to light some essential difference between the two organisms. Bacteriostatic tests were performed by adding to 17 cc of peptone-free, 25% serum, infusion broth 2 cc of sulfanilamide to make a final concentration of 1:10,000. The medium was freshly prepared and incubated overnight, anaerobically. One cc of a diluted 15-hour broth culture was added with the introduction of as little oxygen as possible. Platings were made after 2, 4, 5, 6, 7, 9, 12 and 24 hours' incubation anaerobically at 37° C. At the same time the gross turbidity was estimated by barium sulfate standards and the average number of cocci per chain determined microscopically. The graphic results appear in Figure I.

Unlike the results of the mouse experiments both strains were inhibited by sulfanilamide in vitro. Since it is well known that in



broth containing this drug streptococci produce long chains, the average number of cocci per chain was determined at the time each plating was made. The error due to interpreting the failure of the organisms to divide as true bacteriostasis would have been slight. however, in this instance. There were only 3 times as many elements in the drug-broth as in the control tubes, while bacteriostasis as determined by platings was in the order of a two-hundred fold difference at 9 hours.

Phagocytic Experiments. The discrepancy between the drugresistance of strain S in vivo and its susceptibility in vitro was attributed to the enormous difference in the number of bacteria used in the two types of experiments. In mice the number was very large; in the test tube it was small. Lockwood¹⁰ has demonstrated the antibacteriostatic effect of large inocula in vitro. Nevertheless, it occurred to us that this strain might show marked resistance to phagocytosis in the presence of sulfanilamide. Therefore, a series of 3 in vitro phagocytic experiments was carried out. Using one set of cultures throughout, the test conditions were varied so as to include the use of organisms previously exposed to 1:10,000 sulfanilamide and others not subjected to the drug. The tests were incubated in the water bath at 37° C. with constant rotation. Smears were made after 30 minutes and one hour. Phagocytic activity was estimated by counting 200 leucocytes stained by Wright's method and by determining microscopically the average number of cocci per phagocyte.

A detailed description of the technique is not warranted since it was found that neither strain was markedly influenced by the presence of the drug. Strain 1097 was, perhaps, more readily phagocytized, with or without the drug, than was the S strain.

Further attempts to differentiate strains S and 1097. Because the results in mice had indicated that the strains behaved differently toward sulfanilamide, a series of fermentative tests and dehydrogenase determinations was conducted. The fermentative capacities were similar, however, and dehydrogenase studies, patterned after MacLeod were essentially negative.

Adaptation to aërobic cultivation. After 14 and 18 months respectively strains S and 1097 both developed spontaneously the ability to grow on aerobic blood agar. Since this adaptation must have been accomplished through the acquisition of new respiratory systems, it was thought desirable to determine whether a change in

¹⁰ Lockwood, J. S., J. Immunol., 1938, 35, 155.

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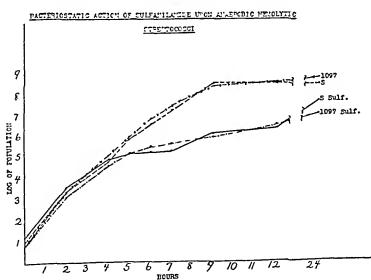


Figure I

11445

Serum Phosphatase, Calcium and Phosphorus Values in Infancy.*

Donald J. Barnes and Bertha Munks. (Introduced by Arthur H. Smith.)

From Harper Hospital, Detroit, Mich.

Investigators who have been studying diagnostic procedures and standards for judging the healing or development of early rickets in infants have commented on the difficulty of making accurate judgments when these must be based upon the physical findings, roentgenological examinations, the serum calcium and serum phosphorus determinations. We have previously cited our belief that, through the determination of the serum phosphatase, we have a more accurate means of recognizing the early development of the disease. In this we simply agree with other investigators2, and offer additional supporting evidence. However, with our interest in this abnormal state in the infant, we were struck by the lack of any considerable data defining the normal serum phosphatase for the infant in the first year of life. The data of Jeans and Stearns4 show that the plasma phosphatase, which is low at birth, rises abruptly to a maximum during the first month, maintains this peak only a short time. and then falls rather rapidly during the second or third month, gradually declining through the remainder of the year, although the phosphatase level remains higher than that found in older age groups.

Our data, based on 630 observations made upon infants during the first year of life, do not entirely coincide with those of Jeans and Stearns but we feel that they represent a good sampling of population of this age for this section of the country. Part of the patients, differentiated as "Harper Babies", upon whom 390 observations were made, were born at Harper Hospital and were followed in our outpatient clinic. They received adequate amounts of milk, vitamin D

^{*} This study was made possible by a grant from the Upjohn Company. The assistance of the Department of Obstetrics is also acknowledged.

¹ Barnes, D. J., and Carpenter, M. D., J. Pediatrics, 1937, 10, 596.

² Bodansky, A., and Jaffe, H. L., Arch. Int. Mcd., 1934, 54, 88; Am. J. Dis. Child., 1934, 48, 1268.

³ Morris, N., Stevenson, M. M., Peden, O. D., and Small, J., Arch. Dis. Child-hood, 1937, 12, 45.

⁴ Stearns, G., and Warweg, E., J. Biol. Chem., 1933, 102, 749.

drug susceptibility had likewise occurred. Therefore, the mouse tests were repeated using aërobic broth cultures as inoculum. Mouse-virulence was again attained with considerable difficulty and each strain tested in 30 mice receiving 20 mg of sulfanilamide per day and approximately the same number of lethal doses of culture as in Table I. No animal survived beyond the sixth day. Infection with strain S, which previously had been resistant to therapy, was now slightly less refractory. On the other hand infection with the 1097 strain, moderately susceptible when produced by the anaërobic variant, became definitely more resistant, so that the results with both strains were strikingly similar. It would appear, then, that the inability of streptococci to grow on aërobic blood agar may not necessarily be correlated with drug resistance in mice. In fact, strain 1097 was more susceptible to sulfanilamide when it was an obligate anaërobe.

Discussion. Bliss, Long and Feinstone⁸ record anaerobic non-hemolytic streptococci as refractory to sulfanilamide both in vitro and in vivo. This opinion is in agreement with the clinical experience of Colebrook and Purdie.⁹ The results with strain 1097 may be of interest, then, by suggesting the possibility that at least some strains of anaerobic hemolytic streptococci are amenable to sulfanilamide therapy.

The in vitro experiments of Shinn, ct al.,4 with Type I pneumococcus show that the gradual reduction of the oxygen concentration to 0.04% is accompanied by a corresponding decrease in bacteriostasis by sulfanilanide. With further reduction of oxygen, however, growth inhibition reappeared. It is possible that a similar mechanism is operating in our tests with the 1097 strain. By the same token strain S may possess a different respiratory mechanism making it resistant to the drug.

Summary. 1. Two weakly virulent strains of "anaērobic," Group A, hemolytic streptococci were subjected to in vivo and in vitro tests with sulfanilamide. 2. One strain was resistant, the other moderately susceptible, to the drug in mice. 3. No essential difference between the strains could be demonstrated, however, by in vitro bacteriostatic, phagocytic and biochemical tests. 4. Following adaptation to aërobic incubation (14 and 18 months) both strains were refractory in mice. 5. The results indicate that anaērobiosis, per se, was not the fundamental factor in determining drug response of these "anaērobic" hemolytic streptococci.

⁸ Bliss, E. A., Long, P. H., and Feinstone, W. H., So. Med. J., 1938, 31, 303.

⁹ Colebrook, L., and Purdie, A. W., Lancet, 1937, 2, 1237.

11445

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^a Morris, N., Stevenson, M. M., Peden, O. D., and Small, J., Arch. Dis. Child-hood, 1937, 12, 45.

⁴ Stearns, G., and Warweg, E., J. Biol. Chem., 1933, 102, 749.

as cod liver oil, and accessory foods as their ages warranted. Where there was any evidence of developing anemia, iron was added to the dietary. Any infants who showed signs or symptoms of rickets, either physical or as judged roentgenologically, were dropped from the group. The average serum phosphatase from these patients did not show the peak rise during the first month noted by Jeans and Stearns.

A second group of patients, known as "Welfare Babies", on which 240 of the observations were made, were selected from those sent to our clinic from Child Welfare stations because they were thought to be rachitic and in need of treatment. From the large number sent, those were selected who were roentgenologically negative and

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	4.4	Harper bab	ies''	"Welfure bables"			
Age groups	No.	Mean, mg per 100 cc serum	Standard deviation, mg	No.	Mean, mg per 100 cc serum	Standard deviation, mg	
	(n)	Statistical	exaluation of	ealeium dat	9.		
0 - 3 days	20	11.3	.83	· · · · · · · · · · · · · · · · · · ·	•••		
3 -15	17	10.8	1.09				
12- 112 mo	31	12.1	.70	8	11,9	.60	
114. 21/2,	28	11,6	.71	30	11.9	.67	
214. 314 "	28	11.9	.62	27	11.9	.69	
316. 414	33	12.1	1.09	23	12.1	.92	
414. 515 "	34	11.8	.66	23	12.1	.77	
514. 614 "	27	11.9	.63	21	12.0	.63	
616. 716 "	26	11.8	.92	28	11,5	.74	
714. 814 "	31	11.9	.79	21	11.9	.59	
816. 914 ''	25	11.9	.67	15	11.9	1.13	
ดิร์รู้ เกรรู้ "	19	12.1	.64	18	12.0	.62	
1016.1116 "	13	11.8	.58	12	12.1	.50	
1134-1214	22	11.9	.65	10	12.3	.61	
1172-15-2		11.7	.007	10	2000	•~-	
Total	354			236			
3 (1/41)		ntistical av	aluation of p		1111		
0 - 3 days	4.5	6.4	.99				
0 - 3 days	23	6.8	.75				
14- 114 ma	33	6.4	.56	8	6,6	.58	
114. 214 "	30	6.7	.78	30	6.3	.58	
214. 314 ''	28	6.5	.77	27	6,3	.52	
31/6. 41/2 "	33	6,3	.93	23	6.1	.51	
414-514 "	34	6.3	.71	23	6.4	.51	
	27	6.4	.61	22	6.2	.51	
970 070	26	6.2	.51	28	6,3	.42	
61/4 71/4 ''	31	5.9	.62	21	6.1	.45	
1 1/2. 0.4	26	ž.š	.59	15	6.2	.49	
872 972	19	6.1	.54	21	6.1	.43	
972-1079	13	5,9	.87	12	6.4	54	
103/2-113/2	22	5.8	.39	10	6.4	.59	
111/2-121/2		2,12	****				
Total	390			240			
				***********	ioo ec. and	a serum	

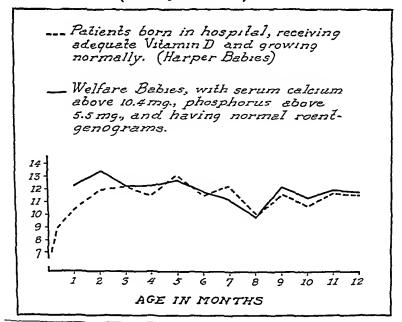
^{*}Patients had a serum calcium of at least 10.4 mg per 100 cc, and a serum phosphorus of 5.5 mg or higher.

who were found to have a serum calcium of at least 10.4 mg per 100 cc and a serum phosphorus of 5.5 mg or above, values which are considered well above the minimal normal levels. From this group, consisting of colored and white babies, we obviously could not get data at birth and relatively few were seen during the first month. Some of them had had vitamin D, generally in small amounts.

The serum calcium, phosphorus and phosphatase were determined on blood drawn from the femoral vein. Clark and Collip's modification of the Kramer, Tisdall method⁵ was used for calcium determinations, and Bodansky's method⁶ for phosphorus and phosphatase determinations.

Table I shows that the calcium values from birth to 3 days for "Harper Babies" fell from an average of 11.3 mg per 100 cc serum to 10.8 mg during the second week, then rose to an average of 12.1 mg at the first month and were maintained rather consistently through the year. Phosphorus values averaged 6.4 mg per 100 cc serum for birth to 3 days of life and rose to an average maximum

SERUM PHOSPHATASE VALUES DURING INFANCY (units per 100 cc.)



⁵ Clark, E. P., and Collip, J. B., J. Biol. Chem., 1925, 63, 461.

⁶ Bodansky, A., J. Biol. Chem., 1932, 99, 197; ibid., 1933, 101, 93.

as cod liver oil, and accessory foods as their ages warranted. Where there was any evidence of developing anemia, iron was added to the dietary. Any infants who showed signs or symptoms of rickets, either physical or as judged roentgenologically, were dropped from the group. The average serum phosphatase from these patients did not show the peak rise during the first month noted by Jeans and Stearns.

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TABLE I.

Age groups ca 0 · 3 days 3 · 15 '' 1 14. 114 mo 3 114. 214 '' 2 214. 314 '' 2 315. 414 '' 3 414. 514 '' 3 414. 514 '' 3 514. 614 '' 2 614. 714 '' 2 714. 814 '' 3 814. 914 '' 2 914.1014 '' 1 1014.114 '' 1 1114.1214 '' 2	Mean, mg 70, per 100 8es ce serum (a) Statistical 20 11.3 7 10.8		No. eases	Mean, mg per 100 ce serum	Standard deviation, mg
0 · 3 days 3 3 days 3 3 days 3 3 days 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	20 11.3	evaluation o	f calcium dat		6
0 · 3 days 3 3 days 3 3 days 3 3 days 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	20 11.3	.83		ถ.	
14. 114 mo 114. 215 '' 216. 314 '' 216. 314 '' 315. 414 '' 315. 615. '' 516. 615. '' 716. 816 '' 816. 914 '' 916.1015 '' 1015.1115 '' 1116.1216 '' 2	7 10.8		-		
114. 214 '' 224 '' 224 '' 244		1.09	_		
2\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	1 12.1	.70	8	11.9	.80
2\\(\frac{1}{6} \), 3\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	8 11.6	,71	30	11.9	.67
3\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	8 11.9	.62	27	11.9	.69
416- 516 '' 3 516- 616 '' 2 616- 714 '' 2 716- 816 '' 3 816- 916 '' 2 916-1016 '' 1 1016-1116 '' 1 1146-1216 '' 2	3 12.1	1,09	23	12.1	.92
514. 615. '' 2 616. 714. '' 2 714. 814. '' 3 815. 914. '' 2 916.1014. '' 1 1014.1114. '' 1 1114.1214. '' 2		,66	23	12.1	.77
614-714 " 2 714-814 " 3 814-914 " 2 914-1014 " 1 1014-1114 " 1 1114-1214 " 2		,63	21	12.0	.63
716. 816. '' 3 816. 916. '' 9 916.1016. '' 1 1016.1116. '' 1 1116.1216. '' 2		.92	28	11.5	.74
815. 915 '' 2 915.1015 '' 1 1015.1115 '' 1 1115.1216 '' 2		.79	21	11.9	.59
914-1014 '' 1 1014-1114 '' 1 1114-1214 '' 2		.67	15	11.9	1.13
1014-1114 '' 1 1114-1214 '' 2		.64	iš	12.0	.62
111/6-12/6 " 2		.58	12	12.1	.50
_		.65	10	12.3	.61
Total 35			236		
(b)	Statistical ex	caluation of p	phosphorus d	nta.	
0 - 3 days 4		.99	_		
3 -15 '' 2	3 6.8	.75	_		
14- 114 mo 3	3 6.4	.56	8	6.6	.58
114. 21/2 ", 3	0 6.7	.78	30	6.3	.58
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314. 414 " 3	3 6.3	.93	23	6.1	.51
416-516 " 3.	4 6.3	.71	23	6.4	.51
51/2 61/2 " 2"	7 6.4	.61	22	6.2	.51
61/2 - 71/2 " 2	6.2	.51	28	6.3	.42
71/2-81/2 " 3:		.62	21	6.1	.45
81/2 91/2 " 20		.59	15	6.2	.49
916-101/2 " 19		.54	21	6.1	.43
1014-1114 " 15		.87	12	6.4	.54
111/6-12/6 " 25		.39	10	6.4	.59
Total 390	-		240		

^{*}Patients had a serum calcium of at least 10.4 mg per 100 cc, and a serum phosphorus of 5.5 mg or higher.

pointed out that this change in serum phosphatase is probably our earliest reliable sign of rachitic activity. Later on in the infant's life there was not the same relationship between slight physical signs and this evidence of activity. Beginning with the third month and continuing through the year, the phosphatase values for the "Welfare" group correspond fairly closely to those of the "Harper" group. We feel that the "Harper" group represents a sufficiently large, well controlled series so that we have not combined it with the "Welfare" group, rather showing each separately. As would be expected, our standard deviations are smaller in the "Harper" group than in the "Welfare" group.

The findings include both summer and winter values. Among the "Harper Babies," 170 cases tested between June 1 and November 1, constituting summer values, averaged 10.8 units for the year; while 220 cases observed from November 1 to June 1 in the winter grouping, averaged 11.0 units. During the first 6 months of life, the winter values of the "Harper Babies" averaged 1.3 units higher than the summer values, while from 6 months to one year of age, the summer values averaged 1.6 units greater than the winter values. There was not a sufficiently regular distribution of summer and winter cases in the "Welfare" group to warrant such comparisons.

These data represent, first, a series of serum phosphatase, calcium and phosphorus observations upon normal infants (Harper Babies) which should help to establish normal serum phosphatase values through the first year of life; second, a comparison with a group (Welfare Babies) which shows a slightly abnormal elevation of serum phosphatase at the first and second months but which, as judged by other serological and roentgenological standards, was normal. This supports the view that the serum phosphatase elevation is the earliest diagnostic sign of rickets, since this latter group was sent to us representing possible early cases of the disease. Furthermore, the "Welfare" group refutes, since it had such small amounts of vitamin D, the possible argument that the ingested vitamin D which was had in moderate amounts by the "Harper Babies" depressed the level of serum phosphatase below the physiological normal. In addition, further correlated data are presented concerning the serum calcium and phosphorus values during infancy.

of 6.8 mg during the second week, receded slightly with the upswing of serum calcium at one month and then established a fairly constant though slightly falling value during the remainder of the year, to an average of 5.8 mg at 12 months. The "Welfare Babies" gave values which corresponded remarkably closely to those of the "Harper Babies."

The average serum phosphatase of the "Harper Babies", from birth to 3 days of age (Chart), has a value of 7.1 units per 100 cc. It shows no peak during the first month but rather a sharp rise to 11.9 units during the first 2 months, a very slow continuance of this rise through the fourth and fifth months to an average value of 13.0 units and then a gradual, slight decline through the rest of the year to a level of 11.5 units. There is a rather pronounced decline at the eighth month to a value of 9.9 units. The data, representing averages, together with standard deviations for the phosphatase values are shown in Table II,

It is noteworthy that the group labeled "Harper Babies" does not have the high average phosphatase levels at the first and second months reached by the "Welfare" patients. This probably is explained by the fact that the "Welfare Babies" were sent in as being possible cases of active rickets and their serum phosphatase values actually were elevated during the first and second months. In other words, some did have this deviation from the normal. It has been

TABLE II. Statistical Evaluation of Phosphatase Data.

		"	"Harper babies"			"Welfare babies" "			
Age group	s	No.	Mean, units per 100 cc scrum	Standard deviation, units	No.	Mean, units per 100 cc serum	Standard deviation, units		
0 - 3	days	45	7.1	2.66			_		
3 -15	11	23	8.9	2.39		_	~		
14-11/2	mo	33	10.4	3,05	8	12.3	3.84		
11/2- 21/2	"	30	11.9	3.01	30	13.5	3.25		
21/2. 31/2	"	28	12.1	2.52	27	12.3	2.87		
31/2- 41/2	,,	33	11.6	2.76	23	12.2	3.15		
41/2-51/2	11	34	13.0	2.92	23	12.8	2.81		
51/2 61/2	,,	27	11.4	2.88	22	11.5	2.56		
61/2- 71/2	,,,	26	12.3	2.44	28	11.1	2.51		
71/2 81/2	"	31	9.9	2.64	21	9.8	3.29		
81/2 91/2	"	26	11.6	2.58	15	12.2	3.18		
91/2-101/2	,,	19	10.8	3.11	21	11.0	3.58		
10½-11½	,,	13	11.8	2.56	12	12.0	3.84		
11%-12%	,,	22	11.5	2.87	10	11.7	3.21		
Tot	al	390			240				

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11446

Mieromelia in Adult Fowl Caused by Manganese Deficiency During Embryonic Development.

C. D. CASKEY* AND L. C. NORRIS.

From the Department of Pontry Husbandry, Cornell University, Ithaca, N. Y.

Byerly, Titus, Ellis and Landauer¹ and Landauer² reported the occurrence of micromelia of nutritional origin in chicken embryos and in newly hatched chicks. Later Lyons and Insko³ observed micromelia in the embryos and newly hatched chicks of hens fed a diet deficient in manganese which was strikingly similar to that described by Byerly and associates and by Landauer. Lyons and Insko prevented the development of the abnormally shortened leg and wing bones by injecting manganese sulfate into the eggs just before placing them in the incubator.

Caskey, Gallup and Norris¹ prevented the development of the embryonic reduction in bone length due to manganese deficiency by feeding the hens manganous carbonate. They also showed that one of the chief symptoms of perosis, which develops in normal chicks fed a manganese-deficient diet, is a reduction in the length of the leg and wing bones. Caskey and Norris¹ found that perosis fails to develop when normal chicks fed a manganese-deficient diet are injected intraperitoneally with manganous chloride. Lyons and Insko¹ and Gallup and Norris¹ reported that the eggs of hens fed a manganese-deficient diet contain markedly less manganese than those of hens fed an adequate diet.

In further studies conducted at this laboratory it was found that frequently the chicks which hatched from the eggs of hens fed a low-manganese diet (0.00063% manganese) were ataxic. A group of 15 of these chicks, most of which were also micromelic, was placed in appropriate quarters for observation. During the first 8 weeks they were fed an adequate diet composed of 44.73% ground yellow corn, 20% degerminated yellow corn meal, 15% dried skim

^{*} Cooperative G.L.F. Exchange Fellow.

¹ Byerly, T. C., Titus, H. W., Ellis, N. R., and Landauer, W., Proc. Soc. Exp. Biol. AND Med., 1935, 32, 1542.

² Landauer, W., Anat. Rec., 1936, 64, 267.

³ Lyons, Malcolm, and Insko, W. M., Jr., Ky. Agr. Exp. Sta. Bul. 371, 1937, 61.

⁴ Caskey, C. D., Gallup, W. D., and Norris, L. C., J. Nutr., 1939, 17, 407.

⁵ Caskey, C. D., and Norris, L. C., Proc. Soc. Exp. Biol. And Med., 1939, 40, 590,

⁶ Gallup, W. D., and Nortis, L. C., Poul. Sci., 1939, 18, 83.

milk. 10% meat scrap, 5% dehydrated alfalfa meal. 2.5% casein. 1.5% steamed bone meal, 0.5% calcium carbonate. 0.5% iodized salt. 0.25% cod liver oil (400 D per g) and 0.02% manganous carbonate. This diet contained 1.8% calcium, 0.95% phosphorus and 0.01% manganese. After 8 weeks the chicks were fed a somewhat similar diet containing 0.005% manganese.

Eleven of these chicks, 8 females and 3 males, attained maturity and were continued on experiment until approximately 16 months of age. Six of the surviving females and 2 of the males were markedly micromelic at the time of hatching. It was observed that the micromelic chicks at no time showed any apparent recovery from this condition.

Measurements of the shanks, the ulna sections of the wings and of the keels of the micromelic females were made at 11 months of age and compared with similar measurements of normal females of the same breeding and of approximately the same age and weight. No difference between the average lengths of the keels of these groups of hens was found but significant differences were revealed between the average lengths of the shanks and the average lengths of the ulna sections of the wings. A preliminary report of these results together with those on the ataxic condition has been made by Caskey and Norris?

At the conclusion of the experiment the 6 micromelic females and 6 normal females of like breeding and of approximately the same age and weight were killed and their bones dissected and measured. The results of these measurements are given in Table I. A picture of one of the micromelic females is given in Fig. 1.

TABLE I. Effect of an Embryonic Manganese Deficiency upon the Subsequent Bone Development of Chickens.

	L	ength			
Bone mczsured	Normal* hens. em	Micromelief hens. em	Reduction in length.	Significance of difference:	
Sternum Femur Tibia Tarso-metatarsus Humerus Radius Ulna Mctacarpus	13.70 9.14 12.90 8.42 7.84 7.95 7.92	13.60 8.15 10.96 6.87 7.30 6.30 7.17	0.7 10.8 15.0 18.4 6.9 11.0 9.5	332:1 1110:1 4999:1 124:1 557:1 768:1 8:1	

N.H. x R.I.R., avg age 17 mo and avg wt 2034 g.

N.H. x R.I.R., arg age 16 mo and arg w: 2032 g. Odds as determined by "Student's method (Z test).

⁷ Caskey, C. D., and Norris, L. C., J. Nutr., 1939, 17. (Supplement, 16,

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In further studies conducted at this laboratory it was found that frequently the chicks which hatched from the eggs of hens fed a low-manganese diet (0.00063% manganese) were ataxic. A group of 15 of these chicks, most of which were also micromelic, was placed in appropriate quarters for observation. During the first 8 weeks they were fed an adequate diet composed of 44.73% ground yellow corn, 20% degerminated yellow corn meal, 15% dried skim

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⁵ Caskey, C. D., and Norris, L. C., Proc. Soc. Exp. Biol. Ann Men., 1939, 40, 590,

^{. 6} Gallup, W. D., and Norris, L. C., Poul. Sci., 1939, 18. 83.

length of the sternum as a consequence of embryonic manganese deficiency. The difference in the effect of the embryonic manganese deficiency upon the bones of the legs and wings and upon the sternum may be related to the fact that the former undergo considerable calcification during the latter stages of embryonic development whereas the sternum is almost entirely uncalcified at the time of hatching.

Byerly and associates¹ reported that the anterior-posterior axis of the skulls of the micromelic chicken embryos which they examined was markedly shortened. Both this group of investigators and Landauer² observed that some of the affected embryos hatched but Landauer² stated that the head was always normal. It has been observed at this laboratory, however, that some of the newly hatched chicks rendered micromelic by manganese deficiency during embryonic development also possessed brachycephalic heads. Several of the micromelic females which were sacrificed at 16 months of age in order to study the reduction in bone length were still brachycephalic. This indicates that similar to the micromelia due to manganese deficiency the consumption of a diet adequate in manganese after hatching does not promote recovery from the brachycephalism.

Summary. Chicks which are rendered micromelic during embryonic development as a result of manganese deficiency do not recover from this condition when fed a diet adequate in manganese during a period of time greatly in excess of that required for the attainment of maturity.

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Rôle of Vitamin C in Addison's Disease.*

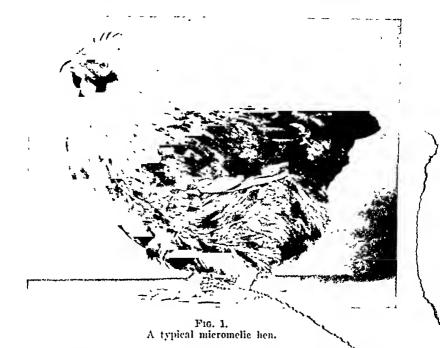
Joseph F. Jenovese, Arnold E. Osterberg and Edward H. Rynearson. (Introduced by J. L. Bollman.)

From the Division of Medicine, Mayo Foundation, Rochester, Minn.

The excretion of ascorbic acid in the urine of patients having Addison's disease has been studied by Siwe, von Drigalski, Geriola, Wilkinson and Ashford. Using the method of Harris and Ray. all the aforementioned investigators found a state of vitamin C

^{*}Abridgement of thesis submitted by Dr. Jenovese to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

MICROMELIA CAUSED BY MIANGANESE DEFICIENCY



The average length of the sterna of the micromelic females was only 0.7% less than that of the normal females. The difference in length was obviously of no significance. The difference between the average length of the metacarpi of the micromelic females and that of the normal females amounted to 8%, but it did not prove to be statistically significant when analyzed by "Student's" method (Z test). All the other differences in bone length proved to be highly significant statistically. The tibiae and the tarso-metatarsi showed the most marked retardation in development, the tibiae being 15% shorter than the normal bones and the tarso-metatarsi 18.4% shorter. The shortening was somewhat greater than that previously reported by Caskey, Gallup and Norris' in newly hatched chicks, but not as great as that reported by Landauer' or that reported by Lyons and Insko' for 21-day embryos and newly hatched chicks.

It is evident from these results that newly hatched chicks which become micromelic during embryonic development as a result of manganese deficiency do not recover from the micromelia when fed a diet containing an adequate amount of manganese during a period greatly in excess of that required for the attainment of maturity. No evidence was obtained, on the other hand, of a reduction in the

TABLE I.

Vitamin C in Blood Plasma and Urine of 6 Normal (Control) Persons; Responses
to Test Dose of Ascorbic Acid.

Control	Date	Vitamin C in mg per 100 M1	Vitamin C 24-hr urinc, mg	Vol., 24-hr urine, Ml	Vitamin C in 3-hr urine, mg, after 500 mg test dosc ascorbic acid	3-lır
1	2-3-39	0.69	18.5	1600	3.6	215
2	1-20	1.15	35.4	800	57.1	110
3	1-21	1.48	58.2	1100	32.2	410
4.	1-20	1.31	30.4	1500	57.1	
5	1-21	0.57	23.8	1210	13.3	350
6	1-23	0.97	24.0	1850	5.8	550

effect on the 3-hour urinary excretion of vitamin C was observed. The blood urea was determined in all cases.

The persons used as normal controls were all young physicians. They gave a history of having been free from recent acute infections or gastrointestinal disorders. The results in each case are contained in Table I.

Methods of Analysis. The method used for the determination of ascorbic acid in the urine was the technic recommended by Harris and Ray; ascorbic acid in the blood was determined by the method of Taylor, Chase and Faulkner. Both of these procedures were described in detail by Magnusson and Osterberg.

Analysis of Results in Controls. The content of ascorbic acid in the blood plasma of the normal persons used as controls ranged from 0.57 to 1.48 mg per 100 MI of plasma. These values are in the normal range as reported by other workers. Similarly, the amounts of ascorbic acid excreted in the urine of the same (normal) persons in 24 hours were normal, varying from 18.5 mg to 58.2 mg (Table I).

The administration of a test dose of 500 mg of ascorbic acid resulted in a 3-hour urinary excretion ranging from 3.6 to 57.1 mg. Two subjects (1 and 6) gave results far below the normal response to be expected from this test (Table I). The other 4 subjects responded normally. Harris and Ray found that the excretion of ascorbic acid in the urine following a test dose of 500 mg of ascorbic acid was usually 8 to 10 times the normal excretion in a 3-hour period.

Experimental Data. Of the 6 patients who had Addison's disease, the patient in case 1 yielded entirely normal results (Table II). In

⁷ Taylor, S. H. L., Chase, Dorrance, and Faulkner, J. M., Biochem. J., 1936, 30, 1119.

⁸ Magnusson, Arlene E., and Osterberg, A. E., Proc. Staff Mect., Mayo Clin., 1938, 13, 700.

deficiency in the cases studied. Wilkinson and Ashford concluded that not only was there a definite deficiency in vitamin C associated with cases of Addison's disease, but that a parallelism existed between the degree of deficiency and the severity of the disease. Sendroy and Miller^a made studies on the combined clearances of urea and ascorbic acid of 8 patients having nephritis and concluded that a relationship existed between the renal efficiency as indicated by the clearance of urea and the amount of ascorbic acid excreted. They suggested that in the presence of Addison's disease in which a functional renal insufficiency exists, the deficiency in the excretion of vitamin C is caused by the renal insufficiency rather than by a state of vitamin C subnutrition. In addition they found a parallelism between the values of urea clearance and the amount of vitamin C excreted in the urine.

The present study was undertaken to investigate further the rôle of vitamin C in Addison's disease by correlating the ascorbic acid content of the blood with the urivary excretion of vitamin C in 6 patients having Addison's disease. The studies on urinary excretion were made according to the method of Harris and Ray, utilizing a test dose of 500 mg of ascorbic acid. The ascorbic acid content in the blood plasma was determined simultaneously with studies of the urine. The blood urea was determined in every instance and, in 4 of the cases, studies of the clearance of urea were made. The investigations were carried out when the patients were first seen and the results reported herein represent the state of vitamin C nutrition that the patients had when they presented themselves for diagnosis. Owing to the fact that the patients were able to stay only a short time, it was possible to carry out saturation studies in only one instance.

Procedure. The subjects were 6 patients with Addison's disease and 6 normal persons as controls. One additional subject with a history of having ingested a diet low in vitamin C for 5 months was also studied. Essentially, the plan of study in each case was to determine first the content of ascorbic acid in the blood plasma in the fasting state. Then the 24-hour urinary excretion of ascorbic acid was determined. To determine the states of vitamin C saturation, the subjects received a test dose of 500 mg of ascorbic acid and the

¹ Siwe, Sture, Klin. Wehnsehr., 1935, 14, 1311.

² von Drigalski, Wolf, Klin. Wehnsehr., 1935, 14, 338.

³ Geriola, F., Minerva med., 1937, 2, 642.

⁴ Wilkinson, J. F., and Ashford, C. A., Lancet, 1936, 2, 967.

⁵ Harris, L. J., and Ray, S. N., Lancet, 1935, 1, 71.

⁶ Sendroy, Julius, Jr., and Miller, B. F., J. Clin. Invest., 1939, 18, 135.

TABLE III.

Data from Detailed Study of Case 6

Date	Vitamin C in plasma, mg per 100 Mi		Vol. 24-br urine. M	Vitamin C in 3 hr prine, mg. after 509 mg test dose assorb c cord	Vol. 3 hr urine, Mi
12 30 3	2.17	7.54	1290		
1-63	9	11.5	1125		
1-9		11.1	1175		
1 10	1.02				
111		11.47	1425		
1 12		15.2	1100		
1-13		9.7	975		
1 13				5.2	100
115		9.2	1050		
1 16		5.4	925		
1 16	(500 mg according acid	intrarenously	7)	151.0	150
115	-	26 a	950	31.7	125
1 19	(500 mg assorbic acid	intravenous';	:		_
	500 mg aerorbic aeid	orall=)		195.7	1125
1.20	(21 br specimen urine c	ontained #45	7 mg m rolu	ne of 1700 MM)	
1 21			~	63 🐇	134
1 26		169	SOG	224	50
1 27	201				
1 30		23 0	1300		
1 31		8.9	1200		
2 1		247	1373		

The observations made in this particular case (Table III) would suggest that perhaps the urinary excretion of vitamin C alone cannot be taken as an adequate criterion for the diagnosis of vitamin C subnutrition. The blood plasma value of the vitamin was normal in all cases studied and yet studies of urinary excretion revealed a low excretion of the vitamin.

Summary and Conclusions The blood plasma values for ascorbic acid in the 6 patients who had Addison's disease that were studied were within normal range. The urinary excretion of ascorbic acid during a 24-hour period was low. On the basis of the results obtained, it would seem that the urinary excretion of ascorbic acid alone cannot be used as an index of vitamin C deficiency in instances of Addison's disease

			TAB	LE	II.	
Data	from	6	Cases	of	Addison's	Disease.

Case	Vitamin C in plasms, mg per 100 M1	Vitamin C 24-hr urine, mg	Vol., 24-lir urine, Ml	Vitamin C in 3-hr urine, mg, after 500 mg test dose ascorbic acid	Vol., 3-hr urine, Ml
1	1,57	20.1	2420	103,50	600
2	0,95	10.4	2730	1.34	365
3	1,22	5.01	1000	33,70	100
4	0.71	13.1	625	2.02	148
ā	0.94	10.0	1100	4.40	417
6	1,15	7.84	1290	4.4	100

this case the history of Addison's disease was of short duration. In addition, there was a history of a more than adequate daily intake of vitamin C in the form of a pint or more of tomato juice.

In the other 5 cases studied (Table II) the ascorbic acid in the blood plasma was also normal or high normal. However, in these cases the amounts of ascorbic acid excreted by the patients in the urine during 24 hours were definitely lower than normal. The values ranged from 5.01 mg to 13.1 mg. According to our normal control subjects and the results reported by others, the normal range of the urinary excretion of ascorbic acid per 24 hours is between 20 and 30 mg. Wilkinson and Ashford reported this same observation on the urinary excretion of ascorbic acid in the cases of Addison's disease that they studied.

The patient in case 6 (Table III) was observed over a longer period than were the other patients. The daily urinary excretion of ascorbic acid of this patient remained low until 500 mg of ascorbic acid was administered intravenously. The excretion value following this procedure increased to 26 mg per 24 hours. days after the initial intravenous dose of ascorbic acid had been administered, 500 mg of ascorbic acid was given orally and 500 mg was administered intravenously. The next 3-hour urine specimen contained 195.7 mg of ascorbic acid and during the following 21 hours 448.7 mg was excreted, making a total excretion of 644.4 mg for the 24-hour period, or approximately 65% of the amount administered to the patient. It would seem that the tissues of this patient must have been saturated with vitamin C to permit such an excretion in 24 hours, yet 5 days after this observation the 24-hour specimen of urine of this patient contained only 16 mg of ascorbic acid. The blood plasma the next day contained 2.01 mg of ascorbic acid, a value which is decidedly above the threshold value of 1.4 mg reported in the literature.

effects of NaCl, KCl, fluids, and adrenal cortical hormone upon rats poisoned with several non-specific toxic agents, including iodo-acetate,

acetate.

Four groups of 10 rats each were divided into 2 subgroups averaging ca 150 g per rat, each group receiving subcutaneously 50 and 80 mg per kg of sodium iodoacetate, respectively. This dosage is distinctly a lower level than the 100-120 mg level employed by Laszt. The rats were fasted and placed on the following ad libitum drinking fluids: (1) tap water, (2) 0.6% NaCl, 0.2% Na Citrate, (3) 0.2% KCl, and (4) tap water. Each group was given by stomach-tube 1 cc per sq decimeter of body surface of the following fluids: (1) tap water, (2) 1% NaCl, 0.1 N NaHCO₂ (ratio of 3:1) or 0.6% NaCl, 0.2% Na Citrate, (3) 1% KCl, and (4) tap water. The latter group was injected subcutaneously with Upjohn adrenal cortical extract, assaying 2.5 Cartland-Kuizenga rat units per cc, at a level of 1 cc per rat at the time of administering iodoacetate, and again 4 hr later. The stomach-tubed fluids were given 4 hours before iodoacetate, at the same time as iodoacetate, and 4 hours before iodoacetate, at the same time as iodoacetate, and every four hours thereafter. Rectal temperatures were taken on all animals every 4 hours, and were seen to drop to approximately 95°F 4 hours after iodoacetate. Eight hours after the 50 mg level of iodoacetate, the NaCl group had nearly regained normal body temperature, with complete return in 12 hours; while all the other groups were still below 96°F at this time. All rats displayed muscular weakness progressing to collapse during the height of the effects of iodoacetate, and marked edema and inflammation at the site of injection, hemorrhagic diarrhea and hematuria. The NaCl group had a marked thirst for the NaCl-Na Citrate drinking fluid,

and after 12 hours of fasting, the group which received the 80 mg level of iodoacetate had gained 37 g, showing a generalized edema.

The groups which received the 80 mg level all died within 8 hours except the NaCl group which survived, with return from 95° temperature to 98° within 20 hours. Potassium chloride decreased survival time, while cortin had no beneficial effects as compared with the controls on tap water. Another experiment, using 12 rats, was performed, in which survival times of cortin-treated and control iodoacetate-poisoned rats were compared for survival. More cortical extract was used than in the previous experiment (Wilson extract, assaying 1 d'Armour unit per 0.1 cc, 0.2 cc per 100 g body weight. Rats weighed av. 325 g), and was given at the time of administration of iodoacetate, and again 6 hours later. No effects

⁵ Cartland, G. F., and Kuizenga, M. H., J. Biol. Chem., 1936, 116, 57.

11448

Effects of Salts and Adrenal Cortical Extracts upon Toxicity of Drugs.

WILLIAM G. CLARK AND RICHARD H. BARNES. (Introduced by Maurice B. Visscher.)

From the Departments of Zoologu and Physiology, University of Minnesota, Minneapolis.

Verzàr and his coworkers¹ in discussing the possible relationships between the adrenal cortex and fat, carbohydrate, and electrolyte metabolism, have postulated that the adrenal cortex controls a wide number of metabolic processes by regulating phosphorylation processes.

One of the primary arguments of these workers is that the effects of iodoacetate poisoning seemed to reproduce some of the symptoms of adrenal insufficiency, such as impaired selective intestinal absorption of glucose, muscular asthenia, steatorrhea, lowered body temperature, fluid loss by diarrhea, etc. These effects are ascribed to a specific inhibitory action of iodoacetate upon phosphorylation.

Laszt has found that NaCl therapy antagonizes the iodoacetate effects in the intact rat, with respect to fatal toxicity, and impaired intestinal glucose absorption. This observation was offered as a possible explanation of some of the beneficial effects of NaCl therapy in adrenal insufficiency.

It appeared possible that this so-called "experimental adrenal insufficiency" produced by iodoacetate, could be explained on another basis than by specific inhibition of phosphorylations. This possibility was brought to our attention by the well known efficacy of NaCl, base, and fluid therapy in many toxic conditions, such as in mercury poisoning, in which fluids are lost by emesis and diarrhea, and uremia ensues as a result of kidney damage. Such toxic agents could not be expected specifically to inhibit phosphorylations under the control of the adrenal cortex, although some of them may produce changes which have been termed symptoms of the "alarm reaction" by Selye and his coworkers. For this reason, we have investigated the

¹ Verzar, F., Die Funktion der Nebennierenrinde, Basel, Benno Schwabe & Co., 1939; Absorption from the Intestine, Longmans, Green & Co., N. Y., 1936.

² Laszt, L., Nature, 1939, 144, 244.

³ Haskell, C. C., Carder, J. R., and Coffindaffer, R. S., J. Am. Med. Assn., 1923, 81, 448.

⁴ Selye, H., Archiv. Internat. Pharm. Therap., 1937, 55, 431; Am. J. Physiol., 1938, 123, 758.

effects of NaCl, KCl, fluids, and adrenal cortical hormone upon rats poisoned with several non-specific toxic agents, including iodo-acetate.

Four groups of 10 rats each were divided into 2 subgroups averaging ca 150 g per rat, each group receiving subcutaneously 50 and 80 mg per kg of sodium iodoacetate, respectively. This dosage is distinctly a lower level than the 100-120 mg level employed by Laszt. The rats were fasted and placed on the following ad libitum drinking fluids: (1) tap water, (2) 0.6% NaCl, 0.2% Na Citrate, (3) 0.2% KCl, and (4) tap water. Each group was given by stomach-tube 1 cc per sq decimeter of body surface of the following fluids: (1) tap water, (2) 1% NaCl, 0.1 N NaHCO₃ (ratio of 3:1) or 0.6% NaCl, 0.2% Na Citrate, (3) 1% KCl, and (4) tap water. The latter group was injected subcutaneously with Upjohn adrenal cortical extract, assaying 2.5 Cartland-Kuizenga rat units⁵ per cc, at a level of 1 cc per rat at the time of administering iodoacetate, and again 4 hr later. The stomach-tubed fluids were given 4 hours before iodoacetate, at the same time as iodoacetate, and every four hours thereafter. Rectal temperatures were taken on all animals every 4 hours, and were seen to drop to approximately 95°F 4 hours after iodoacetate. Eight hours after the 50 mg level of iodoacetate, the NaCl group had nearly regained normal body temperature, with complete return in 12 hours; while all the other groups were still below 96°F at this time. All rats displayed muscular weakness progressing to collapse during the height of the effects of iodoacetate, and marked edema and inflammation at the site of injection, hemorrhagic diarrhea and hematuria, group had a marked thirst for the NaCl-Na Citrate drinking fluid, and after 12 hours of fasting, the group which received the 80 mg level of iodoacetate had gained 37 g, showing a generalized edema.

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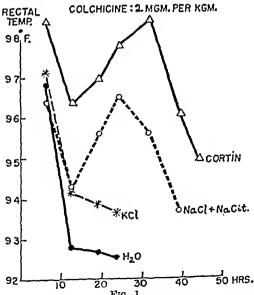
⁵ Cartland, G. F., and Kuizenga, M. H., J. Biol. Chem., 1936, 116, 57.

on survival were obtained, thus confirming the previous result that cortin seems to have no beneficial effect on iodoacetate-poisoning.

Colchicine has been used by Leblond and Sagal[®] in a study of Selye's alarm reaction in rats. There are, however, no reasons to believe that this drug is a specific inhibitor of phosphorylations.

Preliminary experiments indicated that the minimal lethal dose of subcutaneously administered colchicine is less than 1 mg per kg, death being delayed. Food was offered ad libitum in the experiments with colchicine. Pathological symptoms were: collapse, weakness, lowered body temperature, hemorrhagic diarrhea and hematuria, loss of muscular tone, and poor muscular control.

Two levels of colchicine were given, 1 and 2 mg per kg, and essentially the same types of salt and fluid medication as described for the iodoacetate experiments. On the 2 mg level, in contrast to the iodoacetate results, KCl and cortin, as well as NaCl, exerted a beneficial effect as compared with the controls, with respect to body temperature, general activity, and survival. NaCl and cortin, especially the latter, exerted more marked effects. Fig. 1 illustrates the effect of NaCl and cortin on body temperature. It is noticed that the NaCl and especially the cortin groups nearly regain normal



Effect of H₂O, NaCl-citrate, KCl, and Cortin Administration on Body Temperatures of Colchicine-Poisoned Rats.

⁶ Leblond, C. P., and Segal, G., Compt. Rend. Soc. Biol., Paris, 1938, 128, 995.

temperatures, although the effects were not permanent at this level of colchicine. At the lower level of colchicine, the same results were obtained, except that several of the NaCl, KCl, and cortin animals survived indefinitely, whereas all the controls died.

Preliminary results demonstrated a minimal lethal dose of 10 mg per kg of subcutaneously administered HgCl₂ in rats. Death was markedly delayed, hence food was offered. Four groups of rats, averaging ca 240 g, were injected with 20 mg per kg of HgCl₂, thus a lethal dose. The groups were treated essentially as in the previous experiments, except that the hormone was given as subcutaneously administered desoxycorticosterone acetate in oil ("Doca") instead of cortical extract. One mg in oil was injected 0, 12, 24, 34, 43, 43 hr, and 2 mg at 51, 58, and 68 hr. The stomach-tubed fluids were administered at 24, 30, 35, 45, 60, 69, 80, 92, 104, 164, and 192 hr. Potassium chloride was definitely toxic to HgCl₂ poisoned rats, while "Doca" had no effect. The general symptoms were quite similar to those in iodoacetate poisoning except that death was delayed. All but one of the NaCl group had nearly regained normal weight within 2 weeks, and survived indefinitely. All others died; the KCl group first, followed by the "Doca" group, then the controls.

In summary, Fig. 2 shows the pooled results, with respect to survival times of rats poisoned with all 3 toxic agents. The top row of figures shows indefinite survival in all groups on the 50 mg level of

TOXIC AGENT	MED	ICATI	ON A	AND AV	/. HF	S. SUF	RVIV	AL	MLD. MGM.		
MGM, PER KGM	NO. RATS	н ₂ 0	NO. RATS		NO. RATS	KCl	NO. RATS	CORTIN	PER KGM.		
1AA 50	5	8	5	∞*	5	8	5	8			
AAI 80	11	8	5	8	5	5	8	7	>50 <80		
Colch.	4	62	4	2-∞ 2-75	4	2-∞ 2-66	4	1-∞ 3-75	< 1		
Colch.		20	4	28	4	30	4	43	,		
HgCl ₂ 20	13	89	7	6-∞ 1-144	7	43	7	79 [*]	10		

*NaCl +NaHGO3

* DESOXYCORTICOSTERONE ACETATE

Effect of HaO, NaCl, KCl, and Cortin Administration on Survival of Iodoacetic-, Colchicine-, and HgCla Poisoned Rats. on survival were obtained, thus confirming the previous result that cortin seems to have no beneficial effect on iodoacetate-poisoning.

Colchicine has been used by Leblond and Sagal^a in a study of Selye's alarm reaction in rats. There are, however, no reasons to believe that this drug is a specific inhibitor of phosphorylations.

Preliminary experiments indicated that the minimal lethal dose of subcutaneously administered colchicine is less than 1 mg per kg, death being delayed. Food was offered ad libitum in the experiments with colchicine. Pathological symptoms were: collapse, weakness, lowered body temperature, hemorrhagic diarrhea and hematuria, loss of muscular tone, and poor muscular control.

Two levels of colchicine were given, 1 and 2 mg per kg, and essentially the same types of salt and fluid medication as described for the iodoacetate experiments. On the 2 mg level, in contrast to the iodoacetate results, KCl and cortin, as well as NaCl, exerted a beneficial effect as compared with the controls, with respect to body temperature, general activity, and survival. NaCl and cortin, especially the latter, exerted more marked effects. Fig. 1 illustrates the effect of NaCl and cortin on body temperature. It is noticed that the NaCl and especially the cortin groups nearly regain normal

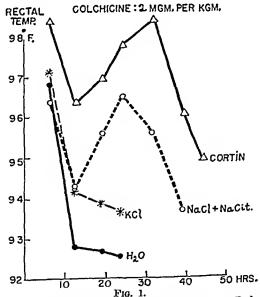


Fig. 1. Effect of H₂O, NaCl-citrate, KCl, and Cortin Administration on Body Temperatures of Colchicine-Poisoned Rats.

⁶ Leblond, C. P., and Segal, G., Compt. Rend. Soc. Biol., Paris, 1938, 128, 995.

11449 P

Transfer of Radioactive Sodium Across the Placenta of the Cat.*

LOUIS B. FLEXNER AND HERBERT A. POHL. (Introduced by C. G. Hartman.)

From the Department of Anatomy, The Johns Hopkins University.

Radioactive sodium, Na24, was prepared by use of the electrostatic pressure generator of the Department of Terrestrial Magnetism, Carnegie Institute of Washington. Samples emitting about 105 beta-rays per second were injected intravenously, as the chloride, into pregnant cats. At various intervals of time after injection. fetuses were removed by Caesarian section and a sample of blood taken from the mother. The radioactivity of the samples, in terms of heta-particles per second, was measured by a pressure ionization chamber and string electrometer, using the method previously described 1

Typical data are presented in Tables I and II. Analysis of these data reveal the following relationships:

- 1. Table I. The fetus near term comes to within 10% of a limiting equilibrium value with respect to sodium ion in the maternal plasma only after 12 to 18 hours. This is in striking contrast to the extracellular fluid of the mother which comes to the same value in about 4 minutes.2
- 2. Table II, column 6. The rate of transfer across the placenta per unit weight of placenta is very low in early stages of pregnancy (gestation age, 15 to 20 days) but increases in linear manner to a

TABLE I.

Delivery time of fetus after injection of Naos.		Betas/see/	Betas/sec/	
hours	Fetal wt, g	total fetus*	g fetus	
1.0	132	2.11	.0160	
6.7	116	11.9	.102	
19.0	96	19.5	.206	
23.5	130	26.0	.200	

^{*}In each instance the number of beta-particles per second emitted by the sample (in all directions) has been corrected for background and radioactive decay and to unit activity of the maternal blood plasma. This makes the data from the several experiments immediately comparable.

These fetuses have a gestation age of 55 days or over.

² Heresy, G., J. Chem. Soc., 1939, 1939, 1213.

^{*} Aided by a grant from the Rockefeller Foundation Fluid Research Fund of the School of Medicine, Johns Hopkins University.

¹ Flexner, L. B., and Roberts, R. B., Am. J. Physiol., 1939, 128, 154.

iodoacetate. The second row shows the marked life-maintaining effect of NaCl therapy in the case of lethal doses of iodoacetate, and lack of effect of cortin, while KCl seems somewhat toxic. The fourth row illustrates the beneficial effects of cortin in fatal colchicine poisoning. The last row of figures illustrates the toxicity of KCl, benefits of NaCl, and lack of effects of "Doca" in mercury poisoning. Temperature trends showed the same results as survival data, and in some cases revealed beneficial or detrimental effects much better throughout the entire study, except in the case of mercury poisoning.

Shorr, Barker, and Malani questioned the specific inhibition of phosphorylation by iodoacctate during glucosc oxidation. Wertheimer, Klinghoffer, Öhnell and Höber, 10 and Doty and Eaton, 11 have stated that iodoacetic acid has no effect upon intestinal absorption of sugar, salts, and amino acids by any specific inhibitory action, but does so if present in grossly pathological quantities, in which the toxic symptoms described in this paper would result. Most workers have used much larger doses than we have employed. Verzar and his coworkers have used impaired intestinal glucose absorption as a criterion of adrenal insufficiency, and have extended reasoning obtained in such experiments to other experiments in which phosphorylation in general is claimed to become impaired in adrenalectomized animals. The data we have presented in addition to the quoted references seem to us to constitute an argument against comparing the non-specific pathology of iodoacctate poisoning with the symptoms seen in the adrenal insufficiency syndrome.

⁷ Shorr. E., Barker, S. B., and Malam, M., Science, 1938, 87, 168.

⁸ Wertheimer, E., Archiv. gcs. Physiol., 1933, 233, 514.

⁹ Klinghoffer, K. A., J. Biol. Chem., 1938, 126, 201.

¹⁰ Ohnell, R., and Höber, R., J. Cell. and Comp. Physiol., 1939, 13, 161.

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11449 P

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LOUIS B. FLEXNER AND HERBERT A. POHL. (Introduced by C. G. Hartman.)

From the Department of Anatomy, The Johns Hopkins University.

Radioactive sodium, Na₂₄, was prepared by use of the electrostatic pressure generator of the Department of Terrestrial Magnetism, Carnegie Institute of Washington. Samples emitting about 10⁵ beta-rays per second were injected intravenously, as the chloride, into pregnant cats. At various intervals of time after injection, fetuses were removed by Caesarian section and a sample of blood taken from the mother. The radioactivity of the samples, in terms of beta-particles per second, was measured by a pressure ionization chamber and string electrometer, using the method previously described.¹

Typical data are presented in Tables I and II. Analysis of these data reveal the following relationships:

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- 2. Table II, column 6. The rate of transfer across the placenta per unit weight of placenta is very low in early stages of pregnancy (gestation age, 15 to 20 days) but increases in linear manner to a

TABLE I.

Delivery time of fetus after injection of Na ₂₄ , hours	Fetal wt, g	Betas/sec/ total fetus*	Betas/sec/ g fetus
1.0	132	2.11	.0160
6.7	116	11.9	.102
19.0	96	19.5	.206
23.5	130	26.0	.200

In each instance the number of beta-particles per second emitted by the sample (in all directions) has been corrected for background and radioactive decay and to unit activity of the maternal blood plasma. This makes the data from the several experiments immediately comparable.

These fetuses have a gestation age of 55 days or over.

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- 2. Table II, column 6. The rate of transfer across the placenta per unit weight of placenta is very low in early stages of pregnancy (gestation age, 15 to 20 days) but increases in linear manner to a

TABLE I.

Delivery time of fetus after injection of Naga,		Betas/see/	Betas/sec/
hours	Fetal wt, g	total fetus*	g fetus
1.0	132	2.11	.0160
6.7	116	11.9	.102
19.0	96	19.5	.206
23.5	130	26.0	.200

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These fetuses have a gestation age of 55 days or over.

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11450

Specific Nature of Complement Fixing Antibody in Malaria as Demonstrated by Absorption Tests.

Anna Dean Dulaney and Warren K. Stratman-Thomas. (Introduced by O. S. Gibbs.)

From the Department of Bacteriology and the Department of Preventive Medicine, University of Tennessee College of Medicine.

We¹ have confirmed the demonstration by Coggeshall and Eaton^{2,3} of a specific complement fixation reaction in malaria employing *P. knowlesi* antigens. The parasites were washed as free as possible of hemoglobin and other blood constituents and dried in vacuo. When ready for use a standardized amount was rehydrated with physiological saline, frozen and thawed, and the supernatant fluid used as antigen.

We have tested sera from 83 patients in whose blood malaria parasites were demonstrated. Seventy-two percent gave a positive complement fixation for malaria at some time during the course of the disease. The positive reaction was correlated with the presence or recent presence of demonstrable parasites but not with the number of parasites. Our results show that a positive complement fixation reaction with our parasite antigen is probably diagnostic of malaria. However, a negative reaction does not rule out malaria.

Sera from 134 individuals presumably free from malaria yielded 127 negative and 7 weakly positive reactions read as 1+ or ±. Forty-three of these sera were known to give a positive Wassermann reaction, 40, a negative Wassermann. One in each group gave a weakly positive reaction with the malaria antigen. It would appear that syphilis does not provoke non-specific reactions with the malaria parasite antigen.

We have further shown that Wassermann negative patients who received induced malaria remained Wassermann negative throughout the course of their treatment, even after they had developed a strongly positive reactivity for the malaria antigen.

Absorption experiments also indicate distinct and unrelated antibodies since treatment of serum with either the malaria or Wassermann antigen removes the specifically reacting substance from that

¹ Stratman-Thomas, Warren K., and Dulaney, Anna Dean, Am. J. Trop. Med., in press,

² Eagle, Harry, and Hogan, Ralph B., J. Exp. Med., 1940, 71, 215.

³ Coggeshall, Lowell T., and Eaton, Monroe D., J. Exp. Med., 1938, 67, 871.

value 60 times that of the early stage at a gestation age of 57 days. After this stage, the rate of transfer per unit weight of placenta decreases somewhat until term (62 days).

3. Table II, column 5. The rate of transfer to a unit weight of fetus, however, is high in early stages and falls with the duration of pregnancy. For example, the ratios of rates of transfer for gestation ages of 15-20 days, 40 days and 60 days are 5.5:2.5:1. The relatively high rate of transfer per unit weight of fetus in the youngest fetuses is accounted for by the large size of the placenta compared to that of the fetus (Table II, columns 2 and 3).

TABLE II.

Gestation age days	Fetal wt, g	Placental wt, g	Betas/sec/ total fetus/hr*	Betas/sec/g fetus/lir	Betas/sec transferred/g placenta/hr
15-20	.15	7	0.013	.085	.002
40	15.0	17.7	0.61	.041	.035
50	52.0	13.5	1.20	.023	.089
57	102.0	13.6	1.60	.0157	.118
62	132.0	25.4	2.11	.0160	.083

^{*}Corrected for background and radioactive decay, and to unit activity of the maternal blood plasma as in Table I.

4. Using the data of Coronios,³ a curve has been constructed relating the percentage daily increase in fetal weight to the fetal age. This curve parallels a curve relating the rate of transfer of Na₂₄ per unit weight of fetus to fetal age. It consequently appears that changes in rate of fetal growth in the cat are accompanied by parallel changes in rate of placental transfer per unit weight of fetus.

The study presented here is part of a comprehensive investigation now proceeding on the comparative physiology of the placenta as revealed by radioactive isotopes.

We are indebted to Dean B. Cowie of the National Caucer Institute for making the sodium bombardments with the Carnegie generator.

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The study presented here is part of a comprehensive investigation now proceeding on the comparative physiology of the placenta as revealed by radioactive isotopes.

We are indebted to Dean B. Cowie of the National Cancer Institute for making the sodium bombardments with the Carnegie generator.

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TABLE I.

Effect of Absorption with Wassermann, Malaria, and Normal Monkey Antigens on Reactivity of Serum for Wassermann and Malaria Antigen.

			Dil	utions	of se	rum		
Serum of patient dis.	Antigen	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64
Untreated	ŧ	4+ 4+		4+ 4+		2+	=	=
Diluted 1:2 with saline and filtered	I *		4+ 4+	4+ 3+	3+	2+		~
Absorbed with Kahn antiger diluted 1:2 with saline, filtered	ı, * †		4+ -	4+	3+	1+		~
Absorbed with malaria antigen 1 x (KAb ₁)	* †		3+ 4+		1+		_	~
Absorbed with malaria antigen 2 \ (KAb ₂)	* †			1+ 4+	_	_	-	>
Absorbed with malaria antigen 3 x (KAb ₃)	*					_		~
Absorbed with normal mon- key antigen I v (NAb1)	* †		4+ 4+	4+ 4+	4+ ±	2+ -		-
Absorbed with normal mon- key antigen 2 v (NAb2)	* †		•	4+ 4+	3+ 1+	2+ -		-
Absorbed with normal mon- key antigen 3 v (NAb ₃)	* †		•••		3+	1+		-

^{*}Malama.

the malaria antigen to a significant degree. (2) Absorption with the malaria antigen removes the malaria antibody without removal of the syphilitic reagin to a significant degree. (3) Absorption with normal monkey red cell "antigen" does not affect the reactivity of the serum with either the Wassermann or the malaria antigen. 4. On the basis of this investigation the complement fixation test for malaria would appear to be indicative of the presence of a specific malaria antibody in patients' sera. 5. The syphilitic reagin and the malaria antibody would appear to be distinct entities.

tWassermann.

serum without modification of its ability to fix complement in the presence of the other antigen.

For these absorption experiments sera giving 4+ reaction with both the malaria and Wassermann antigens were used. We followed the procedure employed by Eagle² in absorption experiments on syphilitic sera using Wassermann and Reiter spirochetal antigens.

The 5 sera tested were obtained from patients who had received induced malaria therapy for the treatment of paresis. Sera obtained from these patients prior to the malaria inoculation gave 4+ Wassermann reactions and no complement fixation with the malaria antigen.

Each serum was divided into 5 portions. Part 1 (2 cc) was absorbed with the sediment from 2 cc of Kahn antigen which had been diluted with 3.5 cc of saline and centrifuged. After incubation for 2 hours at 37.5°C and overnight in the ice box 2 cc of physiological saline were added. Removal of the lipoidal particles was accomplished by centrifugation at high speed and subsequent filtration through a micro-Seitz filter. Part 2 (2 cc) was diluted with an equal volume of physiological saline and served as a control for the filtering process to which Part 1 was subjected. Part 3, (2 cc) was combined with an equal volume of the undiluted P. knowlesi antigen, prepared in our routine manner. After incubation for 2 hours at 37.5°, the mixture was centrifuged and the supernatant fluid removed. One cc (KAb₁) was set aside for testing and the remainder used for a second absorption with the undiluted antigen. In some cases this process was repeated again and the final mixture of serum and antigen left in the ice box overnight when it was centrifuged and the supernatant fluid (KAb₁) removed for testing. Part 4 (2 cc) was absorbed 2 or 3 times with an antigen prepared from red blood cells of normal monkeys to rule out species factors which might influence the reaction with the malaria antigen. Part 5 was used as the untreated serum control.

Complement fixation tests on non-absorbed and absorbed portions of the sera were done at the same time with Wassermann antigen and our *P. knowlesi* malaria antigen. The procedure employed for our routine complement fixation tests was followed. Serum and antigen controls were included. Table I gives the detailed results obtained with the serum of patient DIS. Other sera yielded similar data.

These results show: (1) Absorption with the Wassermann antigen removes the so-called syphilitic reagin, responsible for the positive Wassermann test without affecting the reactivity of the serum with

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Effect of Absorption with Wassermann, Malaria, and Normal Monkey Antigens on
Reactivity of Serum for Wassermann and Malaria Antigen.

			Dil	utions	of se	erum		
Serum of patient dis.	Antigen	Undiluted	1:2	1:4	1:8	1:16	1:32	1:64
Untreated	† †	4+ 4+		4+ 4+		2+	_	=
Diluted 1:2 with saline and filtered	l * 1	** *	4+ 4+	4+ 3+	3+	2+ -		_
Absorbed with Kahn antigen diluted 1:2 with saline, filtered	* *	•	4+	4+	3+	1+	_	=
Absorbed with malaria antigen 1 x (KAb ₁)	*		3+ 4+	2+ 4+	1+	_	_	_
Absorbed with malaria antigen 2 \ (KAb ₂)	* †			1+ 4+	_	_	_	_
Absorbed with malaria antigen 3 x (KAb ₃)	*				_	_	_	=
Absorbed with normal mon- key antigen 1 \((NAb_1)	* †		4+ 4+	4+ 4+	4+ ±	2+	=	_
Absorbed with normal mon- key antigen 2 x (NAb ₂)	Ť		•	4+ 4+	3+ 1+	2+	_	=
Absorbed with normal mon- key antigen 3 x (NAb ₃)	*	••••	<i>:</i>		3+	1+	=	_

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tWassermann,

11451 P

Functional Properties of Isolated Spinal Cord Grafts in Larva! Amphibians.*

PAUL WEISS.

From the Department of Zoology, The University of Chicago.

The thesis that the operation of the central nervous system can be satisfactorily explained in terms of fixed neurone arrangements. rests largely on the interpretation of the structural organization of the central gray, as it presents itself in the normal animal. If the central nervous system really owes its fundamental functional manifestations to the minute details of its neurone architecture, any major disorganization of the latter should thoroughly derange the former. Accordingly, a study of the functional capacities of a central nervous system whose anatomical connections have been thrown into confusion promises information of crucial interest. Such a condition can be produced by transplantation.

Such a condition can be produced by transplantation.

Fragments of spinal cord, including several segments, excised from larval salamanders (Amblystoma punctatum) were grafted into the gelatinous connective tissue of the dorsal fin fold. Hosts and donors were of identical age (ca 2 cm in length) and had been in full functional activity for many weeks. In 7 of the 14 animals thus operated a limb was grafted at some distance anteriorly or posteriorly to the cord graft. All grafts became quickly vascularized and well incorporated.

Histological study revealed 3 main changes in the grafted cord fragments: (1) varying degrees of reduction of the gray matter; (2) considerable deformation and disorganization of the surviving portion; (3) outgrowth of bundles of nerve fibers into the surroundings.

The outgrowing nerve fibers form connections with skin, trunk muscles, and in the presence of a grafted limb, also with the latter. The eables connecting cord and limb grafts are always much stronger than other bundles. This fact, highly significant for the interpretation of normal nerve development, suggests that the first pioneering fibers to become attached to the limb thereby acquire some faculty—a kind of "stickiness", as it were—converting them into a preferential contact pathway for later fibers. Arrived

^{*} Aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.

inside the limb, the fibers form abundant and typical connections with muscles and skin.

Within a few weeks of the transplantation these isolated cordlimb complexes begin to exhibit functional activity, in which 3 successive phases can roughly be identified: an early phase of "spon-

taneous" activity; a later phase of responsivity to stimuli applied to the grafted center; a final phase of true reflex responsivity.

The first phase is characterized by intermittent or almost incessant twitching of the limb muscles. The twitches usually appear in spells, starting with irregular fibrillations and gradually building up to violent convulsions. A single fit may last for several minutes. At the peak of activity, the contractions are remarkably well synchronized, the limb executing strong periodic beats, sometimes at fairly regular intervals of the order of one to several seconds. seizures appear no matter whether the animal is at rest or in motion, but are usually more intense following a period of host activity.

During the following weeks the spontaneous bursts become scarcer, with longer periods of inactivity separating the individual fits. During this phase reactions can, however, often be evoked by lightly pressing against the cord graft; the response follows the stimulus with a latency of sometimes more than a second and consists of anything from a single jerk to a seizure of several minutes' duration.

A few weeks later true reflex responses can usually be obtained by tactile stimulation (with cotton fibers) of the skin in the vicinity of the cord graft. In the course of time the stimulogenous area increases. The reflex response consists of a vigorous indiscriminate contraction of all limb muscles with no sign of coordination. The form of the response is essentially constant for a given case, but its size and temporal characteristics vary with the strength and mode of application of the stimulus, as well as with the condition of the host body. While a weak localized stimulus may yield a single twitch, an increase in the strength of the (mechanical) stimulus or spatial summation (stroking) or temporal summation (repetitive touch) all produce a repetitive response, with the after-discharge sometimes lasting for several seconds. Moreover, the excitability of the preparation fluctuates with the condition of the host body: prolonged host activity is invariably followed by a marked increase in the reflex excitability of the cord graft. This host influence on graft excitability is a humoral effect, since direct nerve connections between the central nervous system of the host and the cord graft are lacking.

In cases in which the grafted cord had innervated trunk muscles, the latter showed reactions similar to the ones described for the limb grafts. The contractions of the segmental muscles were always directed towards the site of the cord graft as the center of innervation. As in the limb cases, irregular fibrillations as well as synchronized beats were observed; in a few instances, slow, tonic, contractions were also noted.

The reactions described in the preceding are positively neurogenic manifestations of the isolated cord-limb graft complex itself. As a crucial check against the possible intrusion of host innervation, the host cord was pithed in several specimens, and finally the portion of the back containing the grafted units was completely excised and tested in isolation. Even so, the preparations exhibited the same functional activities as before. In fact, their excitability was even markedly increased.

These observations demonstrate that a fragment of spinal cord, after undergoing a major involution, is still in possession of certain functional properties which, accordingly, can be regarded as the fundamental dynamic properties of a nerve center deprived of its finer structural differentiation. Thus far the following have been observed in our preparations: Spontaneous firing (later subsiding); long latency; synchronization of discharges; after-discharge; repetitive action; reflexivity; spatial summation; temporal summation; fluctuating excitability; fatigue,

The details of the microscopical examination of the grafts will be reported later. The most surprising fact is the presence of abundant sensory fibers, serving as afferent pathways in the described reflexes, despite the fact that only pure spinal cord without primary afferent neurones had been transplanted. Apparently, fiber processes of the spinal gray have connected directly with the skin and become afferent in function, with a concomitant reversal of the sense of their synaptic transmission. The structural disorganization of the gray has presumably weakened the original polarity and irreciprocity of synaptic relations.

11452

Differentiation of Sera of Two Species of Doves and Their Hybrid.*

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Following the studies of Nuttall, the precipitin-reaction has come into wide use as a means of correlating the taxonomic relationships of species with the serological properties of their serum-proteins. For papers citing the numerous reports of work of this nature, the reader is referred to other sources. 2-6 A few workers have employed the method of absorption of precipitins to differentiate in precipitin reactions, the serum-proteins of species that are distinguishable either not at all or with difficulty by direct precipitation. These are summarized by Cumley.7 (Landsteiner and van der Scheers have pointed out limitations in the principles of absorption of precipitins, which affect the interpretation of the results obtained by this technic.) We are familiar with only a few reports 9-11 dealing with the antigenic relationships of the serum of a species-hybrid to that of each of the parents. In the present tests, the serums of the Pearlneck (Streptopelia chinensis), Ring dove (St. risoria) and their hybrids (F₁-P.N./R.D.) were compared.

The precipitins were produced by injecting 0.6 cc of the serum of a species into rabbits 3 times per week until 6 cc of serum had been injected. The immune sera were collected on the tenth and eleventh days after the last injection. For absorptions, the undiluted anti-

^{*} Paper No. 259 from the Department of Genetics, University of Wisconsin. This investigation was supported in part by a grant from The Rockefeller Foundation.

¹ Nuttall, G. H. F., Blood Immunity and Blood Relationship, 1904, Cambridge, The University Press.

² Baier, J. C., Jr., Physiol. Zool., 1933, 6, 91.

³ Boyden, A., Am. Nat., 1934, 68, 516; Sigma Xi Quart., 1936, 24, 152.

⁴ Hicks, R. A., and Little, C. C., Genetics, 1931, 16, 397.

⁷ Hektoen, L., and Cole, A. G., J. Inf. Dis., 1932, 49, 29,

⁶ Wolfe, H. R., Biol. Bull., 1939, 1, 108; Zoologica, 1939, 24, 309.

⁷ Cumley, R. W., Am. Nat., 1939, 73, 375.

⁵ Landsteiner, K., and van der Scheel, J., J. Exp. Med., 1924, 40, 91.

⁹ Ishihara, M., and Misao, T., Jap. J. Gen., 1929, 4, 147.

¹⁰ Kraus, R., and Prizbram, H., Zentralbl. f. Physiol., 1907, 21, 258.

¹¹ Sasaki, K., Jap. J. Zootechu, Set., 1926, 2, 1; Z. f. Tierzuchlung und Zuchlungsbiol. (B), 1937, 38, 361.

serum to one species was mixed with an equal volume of the serum of the other species, and stored at 2-5°C for 24 hours. The process was repeated, using smaller volumes of the absorbing serum, if, after centrifugation of the mixture, the supernatant fluid produced a ring when tested with this serum. For the precipitin-tests, the antiserum was placed in each of a series of capillary tubes of about 2 mm diameter, to a height of approximately 2 mm, and the antigen in its successive dilutions was carefully layered above. The appearance of a ring at the interface within 2 hours was taken as indication of a precipitate.

TABLE I.
Results of Ring-Precipitin Tests, with Auti-Pearlneck and Auti-Ring Dove Sera.

Antiserum		Absorbed by	Antigens	Highest dilution of antigen giving
No.	Antiserum	serum of	tested	a precipitate
1.0.				
3983	Pearlneck		Pearlneck	1:16,384
,,	,,		Ring dove	1:16,384
,,	,,		$F_1P.N./R.D.$	1:16,384
3983	1)	Ring dove	Pearlneck	1:1024
2.7	"	* * * *	Ring dove	None
27	"	**	$F_1P.N./R.D.$	1:32
3983	"	$F_1P.N./R.D.$	Pearlneck	None
,,	11	""	Ring dove	None
"	, ,	٠,	$F_1P.N./R.D.$	None
19883	13		Pearlneck	1:32,768
1,	"		Ring dove	1:32.768
,,	,,		$F_1P.N./R.D.$	1:32,768
19883	,,	Ring dove	Pearlneck	1:2048
11	,,		Ring dove	None
,,	,,	"	$F_1P.N./R.D.$	1:64
19853	,,	$F_1P.N./R.D.$	Pearlneck	None
11	,,	7)	Ring dove	None
,,	,,	,,	F.P.N./R.D.	None
26381	Ring dove		Pearlneck	1:16,384
_00174	,,,		Ring dove	1:16,384
,,	,,		F ₁ P.N./R.D.	1:16,384
26381	,,	Pearlneck	Pearlneck	None
203.51	**	"	Ring dove	1:512
,,	"	"	$F_1P.N./R.D.$	1:128
26381	"	F, P.N./R.D.	Pearlneck	None
20351	,,	11	Ring dove	None
,,	,,	"	F, P.N./R.D.	Noue
28782	,,		Pearlneck	1:16,384
28162	"		Ring dove	1:16,384
"	"		$F_1P.N./R.D.$	1:16,384
	,,	Pearlneek	Pearlneek	None
28752	,,	,,	Ring dove	1:1024
,,	,,	1 >	F,P.N./R.D.	1:128
	, ,,	F ₁ P.N./R.D.	Pearlneck	None
287,52	,,	7,	Ring dove	None
,,	,,	"	F.P.N./R.D.	None
,,				and shoothed

Concentrated antiserum was used in the tests if the antisera were not absorbed. In the absorption-tests, the antisera were diluted in varying degrees, depending upon the amount of antigeu required for absorption.

The results of the tests involving anti-Pearlneck and anti-Ring dove sera, either unabsorbed or following absorption by the serum of the other species or the hybrid, with the various dilutions of the sera of Pearlneck, Ring dove and their F₁ hybrid, respectively, are given in the table. It will be noted that the sera of Pearlneck, Ring dove, and their F₁, respectively, reacted at the same dilution with each of the various unabsorbed antisera, and that no distinction between them could be made by these tests.

However, when anti-Pearlneck serum was absorbed by Ring dove serum, the reagent thus produced precipitated the serum of both Pearlneck and the F₁, but not that of Ring dove. Likewise, following absorption of Ring dove antiserum by the serum of Pearlneck, the test fluid reacted with the serum of Ring dove and the hybrid, but not of Pearlneck. The serum of the species-hybrid invariably showed a precipitate at a lower dilution with the absorbed fluids than did the serum of either Pearlneck or Ring dove with their respective homologous antisera. Therefore, we may reasonably conclude that the serum of the species-hybrid contains a part, possibly all, of the same, or of related proteins that make for the species-specific qualities of the sera of each of the parental species. Additional evidence as to the possible relationship of the serum-proteins of the hybrids with those of each parent is furnished by the reactivity of the antisera for either parent following the respective absorptions by the hybrid serum. As will be noted, such reagents produced no precipitates at all with any of the 3 kinds of serum. Thus, since the hybrid serum by absorption could remove all the precipitius from the antiserum for either parent, it would seem that the antigenic components of the serum of this species-hybrid were very similar to, if not identical with, those of the serum of both parental species.

Investigations are now under way to determine the reactivity of the serum of backcross individuals (to Ring dove), representing the different unit-cellular characters of Pearlneck.¹²

Summary. The serum proteins of two dove species, Pearlneck and Ring dove, and their hybrid were indistinguishable by direct precipitin-tests; i.c. the three kinds of serum reacted at the same dilution with antisera for each of the two species. Following absorption, however, of the antiserum to one species by the serum of the other, a differentiation of the serum-proteins was readily made. The serum of the species-hybrid appeared to possess a combination of the precipitinogens of both parental species.

¹² Irwin, M. R., Genetics, 1939, 24, 709.

Water and Electrolyte Content of Dolphin Kidney and Extraction of Pressor Substance (Renin).

LILLIAN EICHELBERGER, LOUIS LEITER AND E. M. K. GEILING.

From the Lasker Foundation for Medical Research and the Departmen's of Medicine and Pharmacology of the University of Chicago.

Former findings on the distribution of electrolytes in the blood and skeletal muscle² of the dolphin (Tursiops truncatus), together with the variations revealed in comparisons with terrestrial mammals, actuated a similar examination of the kidneys of the dolphin. The regular occurrence of a pressor substance (renin) in the kidneys of terrestrial mammals stimulated the attempt to demonstrate the presence of such a substance in the kidney of this sea mammal. Therefore, the object of this work was: (1) to determine the water and electrolyte content of the dolphin kidney: (2) to establish the presence of a pressor substance (renin) in this kidney; and (3) to compare the results with corresponding data from the dog as a representative land mammal.

Experimental. One whole kidney weighing 230 g was removed from a live young female dolphin, weighing 80 kg, under sodium phenobarbital anesthesia. The kidney was immediately chilled and frozen. After being wrapped in oiled paper, it was placed in a widemouth thermos bottle and shipped by air express from the Marine Studios, St. Augustine. Florida, to our Chicago Laboratories, as described in a previous paper.¹

The dolphin kidney is a compound organ composed of hundreds of small kidney units, each containing a cortex and medulla. Therefore, an aliquot number of units of the whole kidney was taken for chemical analyses, and the remainder for the extraction of the pressor substance. Units amounting to 40 g in weight were analyzed in triplicate for water, fat, chloride, sodium, potassium, calcium and magnesium, using the procedure and methods described by Eichelberger and Bibler.³ These data are given in Table I.

The pressor substance was extracted from 190 g of the kidney units by a method to be described later. To test the pressor activity.

¹ Eichelberger, L., Fetcher, E. S., Jr., Geiling, E. M. K., and Vos, B. J., Jr., J. Biol. Chem., 1940, 133, 145.

² Eichelberger, L., Geiling, E. M. K., and Vos, B. J., Jr., J. Biol. Chem., 1940, 133, 661.

³ Eichelberger, Lillian, and Bibler, Watter, J. Biol. Chem., 1940, 132, 645.

TABLE I. Water and Electrolyte Content of Dolphin Kidney. The values are given per kilo of fat-free tissue.

	H ₂ O g	Fat g	Cl mM	Na mM	K mM	Ca mM	Mg mM
	821.0	11.7	65.3 om 20 no	84.2	56.8	1.53	6.3
	17.11	meys rre	3111 ±0 110	rmai do	28.0		
Mean	802.2	19.7	67.7	82.6	58.3	2.16	5.7
c*	5.6	9.0	5,3	5.8	4.8	0.53	0.5

^{*}Standard deviation.

the purified extract was injected intravenously into an unanesthetized dog and also into a dog under nembutal anesthesia. Blood pressure of the unanesthetized dog was recorded kymographically by means



CHART 1.

Unanesthetized dog. Wt 11.6 kg. Upper record shows time in intervals of 5 seconds. Middle curve represents femoral blood pressure. (1) Normal blood pressure, 154 mm Hg. (2) 4 cc extract from dolphin kidney (equivalent to 20 g fresh kidney tissue) injected intravenously, blood pressure 228 mm Hg. "X" signifies washings of the needle.

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Water and Electrolyte Content of Dolphin Kidney and Extraction of Pressor Substance (Renin).

LILLIAN EICHELBERGER, LOUIS LEITER AND E. M. K. GEILING.

From the Lasker Foundation for Medical Research and the Departments of Medicine and Pharmacology of the University of Chicago.

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Experimental. One whole kidney weighing 230 g was removed from a live young female dolphin, weighing 80 kg, under sodium phenobarbital anesthesia. The kidney was immediately chilled and frozen. After heing wrapped in oiled paper, it was placed in a widemouth thermos bottle and shipped by air express from the Marine Studios, St. Augustine. Florida, to our Chicago Laboratories, as described in a previous paper.¹

The dolphin kidney is a compound organ composed of hundreds of small kidney units, each containing a cortex and medulla. Therefore, an aliquot number of units of the whole kidney was taken for chemical analyses, and the remainder for the extraction of the pressor substance. Units amounting to 40 g in weight were analyzed in triplicate for water, fat, chloride, sodium, potassium, calcium and magnesium, using the procedure and methods described by Eichelberger and Bibler.³ These data are given in Table I.

The pressor substance was extracted from 190 g of the kidney units by a method to be described later. To test the pressor activity,

¹ Eichelberger, L., Fetcher, E. S., Jr., Geiling, E. M. K., and Vos, B. J., Jr., J. Biol. Chem., 1940, 133, 145.

² Eichelberger, L., Geiling, E. M. K., and Vos, B. J., Jr., J. Biol. Chem., 1940, 133, 661.

³ Eichelberger, Lillian, and Bibler, Walter, J. Biol. Chem., 1940, 132, 645.

2 cc of extract made from dog kidney (equivalent to 10 g fresh kidney tissue) the blood pressure rose to 176 mm Hg and was still at 172 mm at the end of 30 min. (2) The control blood pressure of an 8.5 kg anesthetized dog was 110 mm Hg. After the injection of 2 cc extract made from pig kidney (equivalent to 10 g fresh kidney tissue) the blood pressure rose to 240 mm Hg. The blood pressure remained at 170 mm for 15 min., after which another injection of 1 cc extract caused a second rise to 200 mm Hg, which again continued at a level of 170 mm for 15 min. After a third injection of 2 cc extract the blood pressure rose to 190 mm Hg.

Comments. When the content of water and electrolytes in the kidneys of dolphins was collated with comparable data obtained from the kidneys of dogs (Table I), the only difference was the higher water content in the dolphin kidney. The values for sodium and chloride found here, as well as in dog kidneys, are too high to be accounted for by the extra water, assuming that this water is extracellular and contains the concentration of sodium and chloride expected in extracellular fluids. Therefore, it must be assumed that certain cells of the dolphin kidney engaged in the reabsorption of chloride from the glomerular filtrate must contain chloride as well as sodium. Further, the analytical data reflect either of two possibilities: (1) the existence in the lumen of the nephron of an additional fluid phase, varying in composition as it passes down the renal tubules, which must not be different from that found in dogs or else the analytical results would have been decidedly different; (2) if the chloride and sodium concentration representing the extracellular spaces of the kidney is low, as found in the skeletal muscle,² then the sodium and chloride concentration in the contents of the collecting tubules must be considerably higher than that found in the dog.

The potassium figures, as in dog kidneys, indicate that the intracellular phase of the dolphin kidney is relatively small and of the same comparative volume. In view of the complexity of the system and the different kinds of kidneys, it is surprising that the data for dog and dolphin kidneys exhibit the degree of consistency portrayed in Table I.

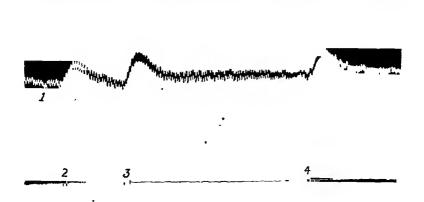
Conclusions. 1. The values for the water content and electrolyte concentrations of a normal dolphiu kidney were as follows: Total water, 821.0 g; chloride, 65.3 mM; sodium, 84.2 mM; potassium, 56.8 mM; calcium, 1.53 mM; and magnesium, 6.3 mM per kg of fat-free tissue.

2. A pressor substance was isolated from the kidney of the dolphin which behaved chemically and physiologically like the renin prepared from the kidney of the dog or pig.

of a gauge 18 needle in the femoral artery connected with a cannula and a mercury manometer. This tracing is shown in Chart I. It will be noted that the control blood pressure of the 11.6 kg dog was 154 mm Hg. After the injection of 4 cc of extract, equivalent to 20 g of fresh kidney tissue, the blood pressure rose to 228 mm Hg. and was still at 212 mm at the end of 10 min.

Blood pressures of the anesthetized dogs were recorded by cannulas in the carotid artery connected directly to a mercury manometer. This tracing is shown in Chart 2 It will be noted that the control blood pressure of the dog under nembutal anesthesia was 152 mm Hg. After the intravenous injection of 2 cc of extract, equivalent to 10 g of fresh kidney tissue, the blood pressure rose to 184 mm Hg. The blood pressure of the dog returned quickly to normal evidently because the amount of extract injected was too small. Another injection of 2 cc of the extract caused a second rise to 194 mm Hg. After a third injection of the extract, the blood pressure rose from 166 mm to 200 mm Hg. and persisted at a level of 172 mm for 10 minutes when the experiment was terminated.

These results show that a pressor substance (renin), which functions like the renin from dog or pig kidney, was isolated from the dolphin kidney. (1) The control blood pressure of a 8.9 kg anesthetized dog was 118 mm Hg. After the intravenous injection of



Anesthetized dog. Wt. 8.2 kg. Records as in Chart 1. (1) Normal blood pressure, 152 mm Hg. (2) 2 ce extract from dolphin kidney (equivalent to 10 g fresh kidney tissue) injected intravenously, blood pressure 184 mm Hg. (3) Second injection of 2 cc of extract, blood pressure 194 mm Hg. (4) Third injection of 2 cc of extract, blood pressure 200 mm Hg.

respectively. The average duration of the fall was 16 days. In the latter 2 dogs, re-implantation of kidney tissue 35 and 330 days after the return of hypertension resulted in a second fall in blood pressure with a return to hypertensive levels persisting for 4 and 36 days respectively after re-implantation. The maximum drop in diastolic pressure amounted to 25 to 50 mm Hg in the 10 experiments and was sustained for the periods indicated. The non-protein nitrogen of the blood remained at normal levels during and subsequent to the depression in arterial pressure,

In 6 normotensive dogs the implantation of kidney tissue had no effect on the blood pressure. The blood pressure remained at the normotensive levels for the duration of the experimental period up to 38 days. Renal ischemia was then produced in 3 of these dogs, 24, 38 and 7 days respectively after the implantation was performed. In all 3 a definite persistent rise in arterial pressure was observed to follow this operation.

In 3 dogs with renal hypertension the production of an infusorial earth abscess had no significant effect on the blood pressure. This abscess was produced by injecting 1 gm of infusorial earth in 10 cc of saline subcutaneously. The large abscess which resulted was opened on the third to fourth day, at which time 25 to 50 cc of pus escaped. The animals improved rapidly following the opening of the abscess.

Our results suggest that during destruction of transplanted kidney tissue, a drop occurs in the blood pressure to normotensive levels in dogs with renal hypertension. No fall in blood pressure occurred following production of an infusorial earth abscess. This is in line with the report⁵ that extensive cellulitis does not lower the pressure of renal hypertensive dogs. Since no fall in blood pressure occurred after implantation into normotensive dogs, the effect seems to be confined to the hypertensive animals. It may persist for long periods beyond the time of removal of the kidney tissue. These facts indicate that the process is not a simple depressor action from absorbed depressor substances but more likely is in the nature of an antagonist to the renal hypertension mechanism. These results are in accord with independent and somewhat different experiments recently reported.^{6,7} A striking difference exists in the two types of ex-

⁵ Wakerlin, G. E., Gaines, W., and Mosny, S. D., Proc. Am. Physiol., Society. New Orleans, p. 191, 1940.

⁶ Harrison, T. R., Grollman, A., and Williams, J. R., Am. J. Physiol., 1940, 128, 716.

⁷ Page, I. H., oral presentation at the American Physiological Society meeting in New Orleans, 1940.

11454 P

Reduction of Arterial Hypertension by Subcutaneous Implantation of Kidney Tissue.*

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We have obtained evidence that the kidney plays an important rôle not only in the production of the hypertension but also in its elimination. To elucidate further the nature of this relationship, we have investigated the effect of subcutaneous implantation of kidney tissue in normotensive and hypertensive dogs.

Blood pressures of trained unanesthetized dogs were taken using the Hamilton manometer,² according to a technique previously described by us.³ After adequate training, the diastolic pressure of most of our dogs became constant at 75 or 80 mm Hg and these were classed as normotensive. Hypertension was induced in 8 dogs by partial occlusion of the main renal arteries, using the Goldblatt technique.⁴

After a preliminary period of observation during which the arterial pressure was relatively constant, 10 to 15 g of kidney tissue obtained from normotensive dogs was implanted under local anesthesia in the subeutaneous tissue of normotensive and hypertensive dogs. On the third day thereafter the necrotic mass of partially autolyzed kidney was expelled from the wound or was removed. Usually 10 or 20 ce of a sero-purulent fluid escaped from the wound at this time. After removal of the necrotic kidney tissue, the animal improved rapidly.

In the 8 dogs with hypertension of renal origin persisting 47, 91, 100, 140, 182, 200, 400 and 29 days respectively, implantation of kidney tissue resulted in a fall in blood pressure beginning on the second day, reaching a low on the third day and returning to the hypertensive level in the fourth to fiftieth days. The blood pressure returned to the hypertensive levels in 1, 50, 20, 4, 5, 7, 3 and 40 days

^{*} Aided by the A. D. Nast Fund for Cardiae Research and the Dazian Foundation for Medical Research.

Now in San Francisco.

¹ Rodbard, S., and Katz, L. N., Am. J. Med. Sci., 1939, 198, 602.

² Hamilton, W. F., Brewer, J., and Brolman, I., Am. J. Physiol., 1934, 107, 427.

³ Katz, L. N., Friedman, M., Rodbard, S., and Weinstein, W., Am. Heart J., 1939, 17, 334.

⁴ Goldblatt, H., Lynch, J., Hanzal, R., and Summerville, W., J. Exp. Med., 1934, 59, 347.

curve. A more extended survey was therefore made of various types of pathological as well as normal bloods, and the polarographic results correlated with variations in the albumin and globulin content as determined by chemical methods. A brief description of the apparatus and the principles underlying its operation has been recently reported by Walker and Reimann.⁴

The blood samples were obtained from fasted subjects. After clotting the serum was withdrawn and aliquots taken for the determination of the serum proteins and for the polarographic test. The serum proteins were determined by Greenberg's colorimetric method. The aliquot used for the polarographic test was treated as follows: 0.1 cc of serum was hydrolyzed by adding 2.5 cc of a 0.05N HC1 solution containing 2.5 mg pepsin and incubating at 40°C for 15 min. 0.1 cc of this mixture was then added to 5 cc of a buffer solution which was composed of 10 cc 1N NH₄OH; 10 cc 1N NH₄Cl; 10 cc 0.01N cobaltic chloride and 10 cc water. Stock solutions of the various constituents of this buffer were kept in separate bottles and were added together in appropriate amounts just prior to each test. The polarographic measurements were made immediately after the test solutions were prepared.

In order to determine which fraction of the serum protein gave the typical curve, the proteins were precipitated from aliquots of serum with (NH₄)₂SO₄ and the precipitate divided into albumin and globulin fractions by solution in dilute salt and by coagulation of the globulin on dialysis against distilled water. The crude protein fractions were then replaced separately, and in combination, into appropriate amounts of deproteinized serum and polarographic measurements made as before. Only the albumin fraction yielded a typical curve; the characteristic waves obtained with intact serum were not observed in the globulin fraction, nor did the addition of globulin materially alter the shape of the albumin curve.

TABLE I.
Relation of Level of Serum Albumin to Polarographic Curve.

		Albu	ımin %		polarographic e in mm
No.	Diseases	Avg	Range	Ārg	Range
7	Nephritis	2.1	1.3-3.1	21.0	13.5-28.0
3	Vomiting of Preg.	2.2	2.0-2.5	22.5	20.5-23.5
3	Osteomyelitis	2.5	1.4-3.6	25.0	13.5-36.0
6	Cancer	2.8	2.5-3.3	26.0	23.0-29.5
22	Miscellaneous*	2.6	1.1-3.8	25.8	17.5-34.5
18	Cardio-vascular	3.0	1.8-4.1	28.0	18.2-34.5
7	Normal	3.5	3.6-4.0	36.5	28.0-44.5

^{*}There were no more than 2 of the same disease in this group.

periments in that no blood NPN elevation occurred in our experiments.

Implantation of liver, skeletal muscle, cardiac muscle, spleen, and boiled kidney had only a transient depressor effect upon the blood pressure of hypertensive dogs.

Experiments are under way to determine the nature of this renal principle and to discover whether or not similar effects can be obtained with other tissues.

Summary. Our results suggest that transplanted kidney tissue undergoing degeneration exerts an antagonistic action upon the renal hypertension mechanism.

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Effect of Serum Proteins on the Polarographic Curve.*

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The first attempt to apply the polarographic method to cancer diagnosis was reported by Brdicka.¹ Since then various others have used this technique as a test for the detection of changes in cancer sera.²-¹ A difference has been demonstrated between normal and cancer blood by these workers, but it appears that this difference is chiefly a statistical one. Our own results fully confirm this difficulty. In a survey of 150 cases, individuals were divided into 3 general groups: normal, non-cancerous diseases and cancer.⁵ The values obtained were expressed as the height of the polarographic curve. There was considerable overlapping in all groups, making it impossible to distinguish any individual case as being representative of normal, cancerous or non-cancerous.

During the course of these studies, serum proteins were determined in some of the specimens which also were examined polarographically. There appeared to be a general parallelism between the amount of the serum protein and the height of the polarographic

^{*} Supported by the Jonathan Bowman Cancer Fund and the Wisconsin Alumni Research Foundation.

¹ Brdička, R., Nature. 1937, 139, 330.

² Bergh, F., Henriques, Q. M., and Wolffbrandt, C. G., Nature, 193S, 142, 212.

³ Wedemeyer, H. E., and Daur, T., Z. f. Krcbs., 1939, 49, 10. 4 Walker, A. C., and Reimann, S. P., Am. J. Ca., 1939, 37, 585.

⁵ Rusch, H. P., Klatt, T., Meloche, V. W., and Dirksen, A. J., in press.

The use of the polarograph as a method for cancer diagnosis depends on the measurement of certain sulfur-containing amino acids present in the proteins in the blood serum. The level of these constituents is reported to differ in normal and cancer sera. Brdicka explains the production of the polarographic curve as being due to the catalytic liberation of hydrogen at the dropping mercury cathode induced by the S-H group of cysteine and the S-S linkage of cystine. While this reaction is said to be catalytic, the amount of hydrogen deposited is quantitatively proportional to the concentration of these amino acids. Brdicka also demonstrated that the cystine group gives a wave height twice that for cysteine when equivalent molecular concentrations are used. 1,6,7 Various workers have determined the cystine content of serum globulin as being about 1.5-3.5% and that of serum albumin from 2,5-6.0%. If we take the usual figures given for the level of albumin as from 4-5 g per 100 cc and those for globulin as 2-2.8 g per 100 cc, we find that the cystine content of the albumin from 100 cc of serum is approximately 0.1-0.3 g while that for the globulin of the same amount of serum is about 0.03-0.09 g. These figures may, in part, explain our findings in regard to the curves obtained with these two separated fractions.

It is interesting to note in passing that various workers have reported a higher sulphydryl content in rapidly growing tissues than in those proliferating slowly.⁸ This has been demonstrated to be the case in embryonic cells, root tips as well as in certain tumors.

Summary. The effect of the serum proteins on the polarographic curve was made on blood sera obtained from 66 normal and pathological individuals. The height of the polarographic curves was found to be directly proportional to the level of the serum albumin. It is obvious, therefore, that the polarographic method is of limited value in cancer diagnosis since changes in serum albumin are by no means specific for neoplastic diseases.

⁶ Brdička, R., Nature, 1938, 142, 617.

⁷ Brdicka, R., Klin. Wochschr., 1939, 18, 305.

⁸ Reimann, S. P., and Hammett, F. S., Am. J. Ca., 1936, 26, 554.

There was a total of 66 cases in this series, the results of which are listed in Table I. When the average results of the serum from various diseases were compared, a direct correlation was found between the level of serum albumin and the height of the polarographic curve. There was, however, a considerable overlapping of the individual cases in each group (Table I). Nevertheless, the individual results also demonstrated a very close parallelism between the amount of the serum albumin and the polarographic response (Fig. 1). Wedeneyer and Daur have reported a similar correlation.³

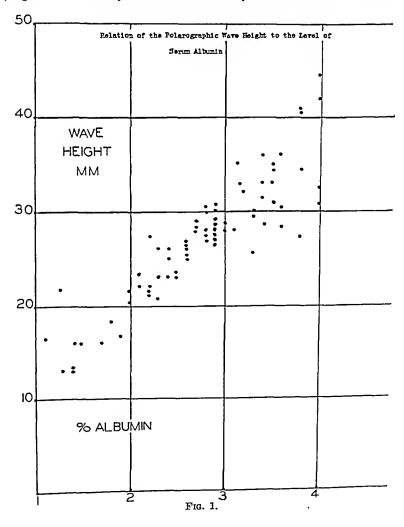


TABLE I. Recovered from Feces During 7-day Absorption Period as Estimated by 3 Chemical Methods.

		Feees wt (dry) for experimental	Recovery furfura	Recovery of pectin by furfural method	necovery o uronic aci	Recovery of pectin by uronic acid method	recovery or pectin by calcium pectate method	ate method
Dog No.	Exp. No.	period, E	ಜ	8	ක	%	20	%
		20 g of	g of pectin per day	lay given with a mix	ted diet.			
н	H 63 E9	151 113	15.24 13.30 11.36	9.50 14.00 8.12 17.40	14.00	10.00 12.42	5.13	3.66
II	H 61 89	145 101	12.68 12.05	9.06 8.62	20.23 15.12	14.45 10.80	10.00	7.14
ш	୮୧୯୯	79 47†	11.71 10.00 6.89	8.37 7.14 6.35	4.33	3.09 1.08	0.00	0.00
Δ	H 01 m	168 95	12.14 12.59	8.68 9.00	20.80 12.22	14.84 8.74	2.19	1.56
Avg		112.4		8.64 Any minon Aminos for	- 7	9.43	4.33	3.09
I	1	197	127.50	91.23	123.80	88.48	120.80	86.30
11	1	188	105.00	75.02	105.30	75.20	95.00	67.80
III	1	484	34.80	24.82	37.40	26.70	32.50	23.06
ΙV		±252	3.15	2.25	4.27	3.05	1.80	1.29
Avg		144	67.86	48.33	62.79	48.36	62.55	44.61

However, 2.345 g of furfural returned, or 11.55%, *(Alenlation: 140 g of pectin was fed, and is represented by 20.3 g of furfural, or 16,24 g of pectin. tGave only one specimen during experimental period.

11456

On the Fate of Ingested Peetin.

S. C. WERCH AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Although pectin is being used for the treatment of certain types of diarrhea, and for other purposes in man, relatively little is known regarding its fate in the alimentary tract. We have been able to find only one report in which the fate of pectin ingested with a mixed diet was studied. Schneider¹ prepared from apple marc a pectin which according to his analysis yielded 35.9% pentosan and 45.8% galactosan. He fed the pectin with a mixed diet very low in cellulose to several human subjects, and found the "coefficient of digestibility" for the pentosans to be 88.7%, and for the galactosans, 76.8%. He also found that intestinal bacteria decompose the hemicelluloses of apple marc. We have studied the fate of pectin fed to dogs with a mixed diet containing no cellulose, and to the same dogs while fasting.

Methods: The pectin used in this study was a very pure citrous pectin obtained from the Research Department, California Fruit Growers' Exchange. By the Link² method 18.2% CO₂ equivalent to 72.8% uronic anhydride was liberated. By the A. O. A. C. method³ for the determination of furfural, 1 g yielded 0.275 g of phlorogluside equivalent to 0.14528 g of furfural. (Uronic acids as well as pentoses yield furfural, hence this factor is not characteristic of all pectins, but must be determined for the particular pectin sample employed.) The methoxyl content was 9.5% and the jelly grade was 180. By the calcium pectate method,⁴ 1 g yielded 110% pectic acid as calcium pectate, on the basis of ash and moisture-free pectin. The foregoing are the average results of a number of analyses made by us. When 5 g of pectin was added to 100 g of feces, 98% could be recovered by the uronic acid method, 94% by the furfural method, and 97.6% by the pectic acid method.

Four dogs weighing from 25 to 35 lb were placed, for the first absorption period, on a mixed diet low in crude fiber (as a control), consisting daily of 200 cc of milk, 200 g of hamburger, and 100 g

¹ Schneider, E. C., Am. J. Physiol., 1912, 30, 258.

² Dickson, A. D., Otterson, H., and Link, K. P., J. Am. Chem. Soc., 1930, 52, 775.

³ Method of Analysis, Association of Official Agricultural Chemists, p. 344, 4th Ed., 1935.

⁴ Joseph, G. H., personal communication.

11457

Exerction of Gonadotropic and Estrogenic Hormones in Urine During Normal Menstrual Cycle.

E. v. HAAM AND N. O. ROTHERMICH.

From the Department of Pathology, Ohio State University, Columbus, Ohio.

None of the methods of bioassay for the urinary gonadotropins or estrogens is better than roughly quantitative. As long as definite standards of technic are not available the various absolute values claimed in the literature cannot be compared and no definite statements as to right or wrong can be issued. However, normal and abnormal fluctuations of these hormones during the menstrual cycle should be recognized with some degree of uniformity regardless of the absolute values obtained by the various methods. Our former belief in a single excretion peak of urinary gonadotropins during the menstrual cycle seems disproven by D'Amour, Funk and Liverman,1 who showed as many as 3 excretion peaks in daily assays of urinary gonadotropins from normal women. Our previous conception of the premenstrual height of estrin excretion has been refuted by the careful investigation of Gustavson and coworkers,2 who showed that as early as 5 days after the onset of menstruction a peak in estrin excretion curve can be found. This demonstrated that more data on the normal menstrual cycle of women are needed in order to establish the correlationship between the excretion of gonadotropic and estrogenic hormones. We have examined the urine of 3 healthy women during a complete menstrual cycle for gonadotropic and estrogenic substances and wish to report briefly on the results:

Method. Twenty-four-hour specimens of urine were collected from 3 women between the ages of 20 to 30 who by careful history did not show any evidence of menstrual disorders. In all 3 women menarche set in between 11 and 13 years, and menstruation occurred every 28 days, lasting from 4 to 6 days. There was no excessive flow, no menstrual pain or premenstrual tension. The specimens were kept on ice during collection and were assayed every 48 hours. Urinary gonadotropins were determined by the method of Levin and

¹ D'Amour, F. E., Funk, D., and Liverman, H., Am. J. Obst. and Guncc., 1939, 37, 940.

² Gustavson, R. G., Mason, L. W., Hays, E. E., Wood, T. R., and D'Amour, F. E., Am. J. Obst. and Gunec., 1938, 35, 115.

of liver. During the second period, 20 g of pectin was added daily. For a third period, the dogs were given pectin alone; 20 g dissolved in 500 cc of water was given by stomach-tube. The absorption periods were 7 days in length. Specimens of feces produced by the dogs during each period were collected, dried and pooled, and pectin or its degradation products determined quantitatively as furfural, uronic, and pectic acids.

When the dogs were fed the basal diet alone, no pectic acid was found in the feces, but a total of from 0.37 to 0.42 g of furfural and from 0.72 to 0.90 g of uronic acid was obtained from the different dogs during the 7 day test period. The quantity of furfural and uronic acid obtained during the control period was subtracted from the total obtained during the pectin-feeding period.

Results: Table I shows the quantity of pectin recovered in the feces as determined by the 3 chemical methods. When pectin was added to the mixed diet, furfural estimation indicated a recovery of 8.64%, uronic acid estimation 9.43%, and pectic acid estimation 3.09% (averages of the 4 dogs.) When pectin was given during fasting, furfural estimation gave a return of 48.33%, uronic acid estimation 48.36%, pectic acid estimation 44.61% (averages of the 4 dogs).

Analysis of the data proved to be very interesting. When one adds pectin to a mixed diet practically 90% disappears, and of the amount recovered in the feces only about a third may be obtained as pectic acid. When given during fasting, about 50% disappeared. In this case, dogs I and II defecated frequently, and the recovery of pectin ranged from about 70 to 90%; whereas in dogs III and IV, which defecated only once during 7 days, the amount of pectin recovered ranged from about 2 to 25%. Essentially the same results have been communicated to us by Drs. L. A. Crandall and H. K. Murer. In addition, the results indicate that the decomposition is carried further when pectin is added to a mixed diet than when given alone, for only about a third of the amount which may be recovered in the feces in the former case may be obtained as pectic acid, while in the latter practically all the pectin recovered may be obtained as pectic acid. Decomposition may be also furthered if the pectin fed is retained for longer periods.

Summary. In the dog when 20 g of pectin was fed per day, with a mixed diet over a 7 day period, an average of 90% of the pectin was decomposed; when fed during fasting an average of only 50% was decomposed. These observations, however, may not be applicable

to man.

11457

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Tyndale,3 using the uterine weight of immature mice as indicators. After precipitation with tannic acid had been completed, the remain-

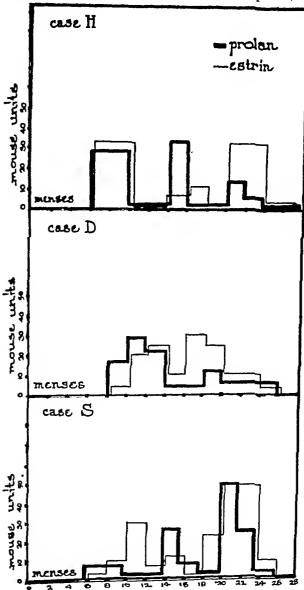


Fig. 1.

Curves of daily excretion of urinary gonadotropins and estrogens during the normal menstrual cycle.

³ Levin, L., and Tyndale, H. H., Endocrinology, 1937, 21, 619.

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Results. The curves depicting the cyclic excretion of estrogen and gonadotropin are pictured in Fig. 1. It appears that 1 to 3 peaks of prolan excretion may occur in a single cycle. The excretion of estrin either parallels or in some instances precedes the prolan excretion. Two distinct peaks have been observed in all our cases. In Patient D they were so close to each other that a separation may seem arbitrary. If we wish to deduce from the appearance of the hormone levels in the urine the time and incidence of ovulation, we must agree with Gustavson's statement that "the time of ovulation may vary considerably in different individuals, and that the corpus luteum may require varying periods of time to reach its full development and activity."

11458 P

Non-Identity of Gray Hair Produced by Mineral Deficiency and Vitamin Deficiency.

ALFRED H. FREE. (Introduced by Victor C. Myers.)

From the Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland.

The recent recognition of a specific organic food factor^{1,2} necessary for the maintenance of the black fur of black or piebald rats has raised the question as to whether the phenomenon of graying noted by older workers^{3,4,5,6} particularly in rats on an exclusive milk diet is due to this cause or is due to a specific mineral deficiency. Jukes and

⁴ Smith, G. V., and Smith, O. W., Am. J. Physiol., 1935, 112, 340.

⁵ Lauson, H. D., Heller, C. G., Golden, J. B., and Sevringhaus, E. L., Endocrinology, 1939, 24, 35.

¹ Morgan, A. F., Cook, B. B., and Davison, H. G., J. Nutrition. 1938, 15, 27.

² Lunde, G., and Kringstad, Z. physiol. Chem., 1938, 257, 201.

³ Hartwell, G. A., Biochem. J., 1923, 17, 547.

⁴ Kiel, H. L., and Nelson, V. E., J. Biol. Chem., 1931, 93, 49.

⁵ György, P., Biochem. J., 1935, 29, 741.

⁶ Gorter, F. J., Z. Vitaminforsch., 1935, 4, 277.

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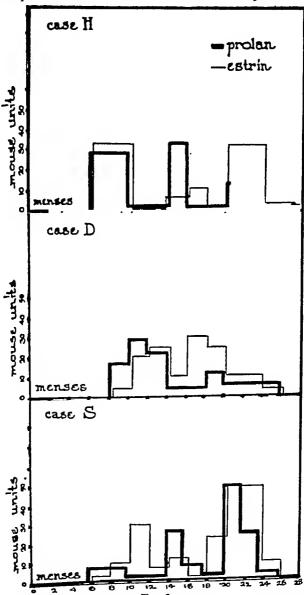


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⁶ Gorter, F. J., Z. Vitaminforsch., 1935, 4, 277.

Richardson⁷ have pointed out that milk is not a good source of filtrate fraction vitamins.

The black fur of young piebald rats fed an exclusive diet of either powdered whole milk or of fresh certified milk rapidly and uniformly became gray. Addition of a supplement of 0.25 mg of iron, 0.05 mg of copper and 0.05 mg of manganese cured the grayness if it had the time of prevented any change in the color of the fur of rats which were fed milk with the above mineral supplements from the time of weaning. Attempts to ascertain which of the 3 metals is responsible have not at present yielded entirely conclusive results.

Rats developing a nutritional anemia show a marked diminution of appetite and the possibility exists that the graying of rats may be due to a diminished intake of vitamin accompanying the reduced intake of the vitamin-poor milk. This has been disproven since in 2 groups of rats fed isocaloric amounts of milk, those supplemented with iron, copper and manganese did not develop grayness whereas those fed milk alone became quite gray. It has also been found that rats that have developed grayness will become black when supplemented with the above minerals whereas the isocaloric controls without minerals remain gray. Rats, following the production of gray hair by a milk diet, when supplemented with a rice bran extract* rich in the factors of the B complex very slowly regained their black color. However, control rats fed equal quantities of the ashed rice bran extract likewise slowly became black. Hemoglobin regeneration slowly occurred in both the rats receiving rice bran and in the rats receiving rice bran ash. This indicates that the effect with milk-fed gray rats is entirely due to inorganic substances in the preparation.

Rats fed a synthetic diet consisting of purified casein, cane sugar, butter, salt mixture, cod liver oil, and supplements of synthetic vitamins B₁, B₆, and riboflavin† developed gray hair. The hemoglobin level in these rats was normal. Iron, copper, and manganese supplements in no way altered the response of these animals. However, rice bran extract in the same amount used above was effective in the cure of these animals. With the vitamin-deficient animals the ash of the rice bran was without effect. Rats given a daily ration of 50 cc of milk plus 1 g of the synthetic diet showed no graying or were cured when placed on such a diet after production of the gray hair either by mineral or vitamin deficiency.

All of the present evidence based on experiments with approxi-

⁷ Jukes, T. H., and Richardson, G. A., J. Agr. Res., 1938, 57, 603.

^{*} Obtained through the courtesy of Dr. Paul György.

t Kindly supplied by Merck and Co., Inc., Rahway, New Jersey.

mately 50 rats seems to indicate that graying of black hair in rats may result from a deficiency of a factor or factors present in the vitamin B complex and may also result from a deficiency of iron, copper, and manganese.

11459

Effects of Testosterone Propionate on Female Roller Canaries under Complete Song Isolation.

Francis Marsh Baldwin, Howard Sidney Goldin and Milton Metfessel.

From the Physiological and Psychological Laboratories, University of Southern California, Los Angeles, Calif.

It is commonly assumed that secondary sexual characteristics are conditioned by the secretions of the respective gonads of each sex. Singing in canaries is normally limited to the male of the species and so may be considered as a male secondary sexual characteristic.

Baldwin and Goldin¹ indicated that when testosterone propionate was administered to the female viviparous teleost, Xiphophorus helleri Heckel, the male secondary sexual characteristics were induced in all cases. Noble and Wurm² treated adult females and immatures of both sexes of the black-crowned night heron, and produced male sexual behavior. They concluded that the differences between the sexual behavior of adults of this form seem to be regulated only by proportionate differences in the amounts of male hormone normally found in these birds. Allee and Collias³ reported crowing in hens treated with testosterone propionate, and cessation of this crowing soon after the treatment was stopped, indicating the dependence of this behavior on the male hormone.

Leonard' treated female roller canaries with testosterone propionate and reported that they produced song that differed from normal male song only in the greater sound volume produced by the males. He also stated that his best results were obtained when the females were "isolated" by putting them in individual cages but keeping them in

¹ Baldwin, F. M., and Goldin, H. S., Proc. Soc. Exp. Biol. AND Med., 1939, 42, 813.

² Noble, G. K., and Wurm, M., Anat. Rec., 1938, 72, Sup. 1, 60.

³ Allee, W. C., and Collias, N., Anat. Rec., 1938, 72, Sup. 1, 60,

⁴ Leonard, S. L., Proc. Soc. Exp. Biol. and Med., 1939, 41, 229.

the same room.* Shoemaker likewise induced song in treated female eanaries, and observed that the failure of these birds to tread receptive females may reflect the lack in testosterone propionate of the eapacity to imitate the complete chain of events caused by the normal testicular hormone, and not to a lack in the nervous system, as two untreated females were observed to copulate like males.

From a behavior standpoint, Lashley indicated that in the rat, hormone action scems to activate some central nervous mechanism to maintain excitability or activity. Moore made observations on eastrated and transplanted rats that showed the transforming power of the gonads of one sex over the psychic nature of the opposite sex. He concluded that this psychic behavior, absolutely distinct in itself, lends great weight to the idea of transformed sexual nature.

Experimental. The canaries used were raised out of doors by a local breeder, and were all past one year of age. Each bird was put in its own soundproofed, ventilated cage8 to preclude any effects of song environment in addition to the administration of the testostcrone propionate. These birds were isolated for obscrvation over a preliminary period of 2 weeks before administration to assure the absence of any song. A crystal microphone was placed in each cage and provision made for recording any sound of the birds on aluminum dises. An operator listened to the sounds at a control board in another room. As soon as any sounds were heard; samples were recorded. Lights were on in the cages at regular daily intervals corresponding in length to the normal waking hours of the birds.

Six birds were used in this problem, 4 for treatment with testosterone propionate, and 2 to aet as oil-treated controls. Injections were made daily at the same hour, each bird receiving 2.5 mg of testosterone propionatet per injection. Thirteen subcutaneous injections in alternate breast regions constituted the extent of the experimentation. The controls were given similar treatment with sesame oil.

Results. While the microphones, amplifiers and recording disc were in readiness for recordings of the voices and calls of the treated birds from the start of the injections, the first song calls in any of the treated birds were forthcoming on the twelfth day. The

^{*} From personal communications with the author, S. L. Leonard.

⁵ Shoemaker, H. H., PROC. Soc. Exp. Biol. and Med., 1939, 41, 299.

⁶ Lashley, K. S., Psycholog. Rev., 1938, 45, 445.

⁷ Moore, C. R., J. Exp. Zool., 1919, 28, 137.

⁸ Metfessel, M., J. Psychology, 1940, 10, 177.

[†] The male hormone, Oreton, was furnished through the kindness of Dr. Max Gilbert of the Schering Corporation.

second bird sang on the thirteenth day, the third bird on the sixteenth day, and the fourth bird on the twentieth day. From these data, it appears that the average length of time in administration of the hormone approximates fifteen days to produce song. Three of the treated birds gradually developed male-like tours that were somewhat of the same pattern and quality, with limited variations. The fourth bird showed a song of a varied pattern that followed the same sequences when the songs were repeated. The songs developed exhibit a small repertoire with poor male quality so far, but with voice that was definitely male in character. Cessation of treatment resulted in a return to the ordinary female calls.

Conclusion. The administration of testosterone propionate to normal adult female roller canaries under conditions of complete song isolation brings forth male-like song in approximately 15 days after first administration, and thus substantiates previous theories.²⁻⁷

11460 P

Isolation of a Murine Neurotropic Virus by Passage of Monkey Poliomyelitis Virus to Cotton Rats and White Mice.*

CLAUS W. JUNGEBLUT AND MURRAY SANDERS.

From the Department of Bacteriology, Columbia University College of Physicians and Surgeons, New York.

Armstrong^{1,2,3} reported apparent transmission of poliomyelitis (Lansing strain) from the monkey to the Eastern cotton rat and to white mice. This report deals with attempts to adapt other strains of poliomyelitis virus to these rodents.

Cotton rats (Sigmodon hispidus littoralis) were infected intracerebrally with 5 recognized strains of monkey poliomyelitis virus (RMV, Aycock, Philadelphia, ST Los Angeles, SK New Haven). None of the animals injected with the first 4 strains showed any abnormal symptoms. However, of 2 cotton rats injected with the SK‡

A tour is somewhat analogous to a syllable of language; there are thirteen recognized tours in roller canary song.

^{*} Sapported by a grant from the Philip Hanson Hiss, Jr., Memorial Fund.

[†] Fellow in Dermatology.

¹ Armstrong, C., Public Health Reports, 1939, 54, 1719.

² Lillie, R. D., and Armstrong, C., Public Health Reports, 1940, 55, 115.

³ Armstrong, C., Public Health Reports, 1939, 54, 2302.

[‡] Received in its 11th monkey passage through the courtesy of Dr. John R. Paul,

strain 1 died the following day, evidently of trauma; the other one succumbed one week later without observed symptoms. No lesions were present except a markedly congested brain, sterile upon aerobic and anaerobic cultivation. Intracerebral transfer of this brain to another cotton rat resulted in mild nervous symptoms within 2 days, and death the next day. Further passage of the brain of the second cotton rat produced in a third cotton rat flaccid paralysis of both hind legs on the 6th day, followed by death 24 hr later. From the last 2 cotton rats intracerebral transfers of brain suspensions were made to groups of white mice. All injected mice developed complete flaccid paralysis of the hind legs, within 3 or 4 days, followed by generalized paralysis and death.

Subsequent attempts to reproduce passage from monkey to cotton rats and white mice with the original material were unsuccessful. Mouse virus, however, since its isolation, is transmissible from mouse to mouse in an unbroken series. At the time of this writing, i. c., April 24th, 1940, the virus is in its 23rd passage. mice have been inoculated; excepting those injected with virus known to be inactivated or impotent all mice have developed the same characteristic symptoms, with only an occasional recovery. to wit: flaccid paralysis (unilateral or bilateral) of hind legs, seldom of forelegs, occasional encephalitic syndrome, death. The described symptomatology is somewhat similar to that of Theiler's spontaneous mouse encephalitis, but the two viruses differ in important aspects (degree of virulence, incubation period, routes of infection, age factor, latent immunization, serological reactions). Moreover, mice from a Theiler-inmune colony are not protected against infection with the mouse virus.

Stained brain suspensions from paralyzed mice, when examined microscopically, show no characteristic morphological unit. Seeding of blood agar, broth or 10% serum broth results in no visible growth after prolonged aerobic or anaerobic incubation. However, the infectious agent passes through V, N and W Berkefeld filters without appreciable diminution in virulence. It is completely destroyed by heating for ½ hr at 60°C and by exposure to ultraviolet light for 1 min, but resists phenol up to 1% concentration. In glycerin it has remained viable up to 1 month in the icebox.

White mice may be successfully infected by any one of the following routes: intracerebral, intranasal, intraperitoneal, intravenous, subcutaneous and by feeding. Upon intracerebral injection a constant potency of 1:1,000,000 is obtained and an occasional endpoint

⁴ Theiler, M., Science, 1934, 80, 122.

of 1:20,000,000. The maximum incubation period has not exceeded 1 week and may be as short as 48 hr with lower dilutions. Intraperitoneal injection of a dose of 1:1000 of a virus brain suspension uniformly produces paralysis within from 3 to 4 days. As early as 2 hr after introducing the virus into the peritoneal cavity it may be recovered from the brain and blood in both of which it persists until the terminal stage. Virus concentrations were highest in the brain and cord, with the adrenal next. Other organs (spleen and liver) carried smaller amounts of the infectious agent.

The virus fails to induce any symptoms in albino rats, guinea pigs and rabbits following repeated injections of large doses by a diversity of routes. It is easily transferable back to cotton rats, producing regularly paralysis and death in that species. Its pathogenicity for monkeys is questionable. Of 10 monkeys injected intracerebrally with either mouse or cotton rat virus 8 passed through a sharp fever cycle; only 5 animals developed weakness of the extremities and 2 others slight transitory facial paresis. In no case did the symptoms progress to typical spinal paralysis.

In the brain of paralyzed mice diffuse proliferation of glia cells

In the brain of paralyzed mice diffuse proliferation of glia cells and occasional foci of perivascular cuffing are observed. Severe damage occurs in the cord in both anterior horns, extending from loss of Nissl substance and irregular nuclear staining to a complete breakdown of the nerve cell with subsequent neuronophagia. Microglial proliferation is widespread at some levels as is perivascular infiltration.

Doses of virus ranging from 1:1,000,000 to 1:100 were tested for *in vitro* inactivation by the following sera: monovalent immune sera from monkeys convalescent from infection with RMV, Aycock, or SK virus, hyperimmune horse serum (anti-RMV), pooled human convalescent serum, normal serum from man, monkey and horse and antiviral immune sera against other neurotropic viruses (Theilermouse encephalitis, equine encephalomyelitis, rabies, St. Louis encephalitis, herpes). Normal animal and human sera, as well as the other antiviral immune sera, failed to bring about inactivation of the virus as its highest effective dilution (1:200,000); convalescent Aycock and SK monkey sera and convalescent human serum neutralized at slightly lower levels (1:100,000 to 1:50,000). Neutralization extending through a virus concentration of 1:1000 was obtained with the hyperimmune horse serum. RMV monkey convalescent serum failed to neutralize. The above data are based on results obtained in repeated tests. These immunological reactions are consistent with those of SK virus in monkeys, which is

neutralizable by SK and Aycock antiserum but not by RMV convalescent monkey serum.⁶ A discrepancy exists regarding normal human serum which neutralizes SK virus in monkeys but not mouse virus in mice (3 sera tested).

Eight monkeys were immunized with a series of subcutaneous injections of live mouse virus and then tested for cross-immunity by intracerebral injection with 3 different strains of virulent monkey poliomyelitis virus. The results of this experiment were as follows: Three immunized monkeys, subsequently infected with the homologous virus, SK, remained free from paralysis of the extremities; none of the other 5 immunized monkeys, subsequently infected with the heterologous strains (Aycock and RMV), escaped the disease. An equal number of controls, infected with the same virus strains, developed typical poliomyelitis. Neutralization tests with monkey sera obtained at the end of immunization showed various titers of mouse virus neutralizing antibodies; in 6 instances neutralization was obtained in monkey tests against SK, Aycock, and RMV virus (3 with SK, 2 with Aycock, 1 with RMV).

In its 6th mouse passage the virus was cultivated in serumultrafiltrate tissue cultures (Sanders⁶) containing embryonic mouse or guinea pig brain or whole minced chick embryo by transferring every 3 days supernatant fluid or whole culture emulsion. The 6th serial passage of embryonic mouse brain cultures produced typical symptoms and death in mice following injection up to 1:10,000,000 dilution of the supernatant culture fluid. Similar passages of guinea pig brain cultures titrated up to 1:10,000. Only traces of virus were recovered from the chick embryo cultures.

⁵ Trask, J. D., Paul, J. R., and Vignec, A. J., Proc. Soc. Exp. Biol. and Med., 1939, 41, 241.

⁶ Sanders, M., J. Exp. Med., 1940, 71, 113.

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Experimental Arthritis in the Albino Rat Produced by a Group A Hemolytic Streptococcus.*

SIDNEY ROTHBARD. (Introduced by E. L. Opie.)

From the Department of Pathology, Cornell University Medical College, New York City.

Collier¹ described a spontaneous polyarthritis in rats from which no microörganism was cultivated. However, bacteriologically sterile organs produced the disease on reinoculation into other rats. Rhodes and van Rooyen² reported a similar disease in rats from which no bacteria were recovered by aerobic or anaerobic methods. Findlay, Mackenzie, MacCallum and Klieneberger³ also described a spontaneous, infectious arthritis in rats, but they were able to isolate a pleuropneumonia-like organism (L7) in pure culture which reproduced the arthritis when injected into other rats. Recently Watson¹ has shown that an acute purulent arthritis can be produced in mice after injections of several strains of hemolytic streptococci.

Because of the interest aroused in this subject by these reports and because a review of the literature reveals no account of the disease in rats due to the streptococcus, it seems advisable to report the production of an acute polyarthritis in the albino rat by the intravenous injection of a hemolytic streptococcus recently isolated from the blood stream of a patient with septicemia.

The streptococcus was of the "matt" variety, Group A and of an unclassified type.† 0.5 cc of an 18-hour broth culture when injected intravenously produced arthritis in 100% of rats weighing from 70 to 100 g while a smaller dose (0.1 cc) produced it in approximately 70% of the animals.

The arthritis appears as early as 48 hours after inoculation. It is multiple in character and new joints develop in succession for 8 days after inoculation. In a few instances the swelling in some joints has decreased during the period of observation while other joints are

^{*} Supported by a grant from the John and Mary R. Markle and the Ophthalmological Foundations.

¹ Collier, W. A., Geneesk. Tijdschr. Ned.-Ind., 1938, 78, 2845.

² Rhodes, A. J., and van Rooyen, C. E., J. Path. and Bact., 1939, 49, 577.

³ Findlay, G. M., Mackenzie, R. D., MacCallum, F. O., and Klieneberger, E., Lancet, 1939, 2, 7.

⁴ Watson, R. F., personal communication, 1940.

[†] Grouped by R. C. Lancefield of the Hospital of the Rockefeller Institute for Medical Research.

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becoming involved. The diseased joints are swollen, dusky red in color, hot and painful to palpation. (Fig. 1.) In some animals as many as 8 different joints develop arthritis. The ankle joint is involved most often, the wrist next in frequency and then the tarsal and carpal interphalangeals. Many of the joints healed completely, but others have progressed, and the arthritis has been present for 8 weeks after inoculation. The rats move about with difficulty, drag their hind limbs and appear ill, but as a rule, do not succumb to the infection.

Gross and microscopic examinations have been made up to 7 days after onset of the disease. At this period the joint is enlarged, the periarticular tissues are oedematous and have a mucinous consistency. The synovial membrane is grey yellow in color and covered with a gelatinous exudate. The synovial fluid is in slight excess, viscid and opaque, but not purulent. The cellular content of the

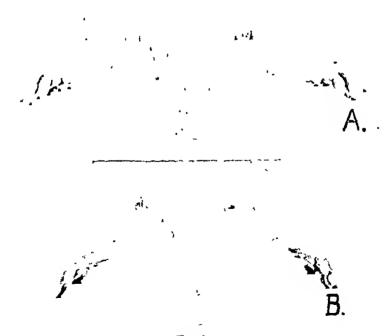


Fig. 1.

Comparison between the ankle joints of a rat with arthritis 7 days after the intravenous injection of 0.5 ee of hemolytic streptococci (A) and those of a normal rat (B).

exudate shows on an average, 65% polymorphonuclear leukocytes and 35% round cells. Streptococci are demonstrable in smears of the synovial fluid and are cultured without difficulty. These organisms, when reinjected into other rats, reproduce the disease. The cartilage and bone show no changes at this stage. Heart blood cultures are positive for 5 days after the intravenous injection. In 5 instances an acute purulent endophthalmitis involving one eye was found

Microscopically, the periarticular tissues are oedematous with separation of the muscle fibres and fascia. Fibrin and pink-staining fluid are present in the interstitial spaces. The tissues are infiltrated with polymorphonuclear leukocytes which are found in focal collections in some areas, a few monocytes and an occasional lymphocyte. The subsynovial fat shows the same type of cellular reaction that is present in the periarticular tissue. The most conspicuous inflammatory reaction appears in the synovial villi. In some areas the synovial membrane is absent, but in others there is evidence of proliferation of synovial cells. The joint cavities contain considerable cellular debris, fibrin, polymorphonuclear leukocytes and round cells.

A more detailed study of this experimental disease is now in progress.

Summary. An acute multiple arthritis has been produced in 45 of 51 albino rats by the intravenous injection of a Group A hemolytic streptococcus. In the eyes of 5 rats, an acute monocular purulent endophthalmitis was present.

The author wishes to thank Dr. D. M. Angevine for his assistance in this work.

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Availability of Glucose for Human Brain Oxidations.*

JOSEPH WORTIS AND WALTER GOLDFARB.

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The ready availability of glucose for brain tissue oxidations has been demonstrated both in the Warburg apparatus (Warburg, Posener and Negelein1) and in the intact organism (Himwich and Nahum,2 Lennox3) Damashek, Myerson and Stephenson4 have shown that when the human brain is deprived of this foodstuff during insulin hypoglycemia the oxygen uptake of the brain is correspondingly diminished, and Himwich, Bowman, Wortis and Fazekas have shown that in the deep coma associated with therapeutic insulin shock brain metabolism approaches zero. Under these conditions in humans the brain potentials have been found to be diminished (Hoagland, Rubin and Cameron^a) and typical neurologic signs appear. Clinical coma, neurologic signs and the characteristic brain potential changes all disappear after glucose administration. The increased oxygen uptake by the brain after glucose administration had already been demonstrated in dogs (Himwich, et al.,7), and in humans the neurological signs are alleviated by the administration of as little as 4 g of glucose (Himwich, Frostig, et al.8)

In the course of our investigations of the availability of various food substrates for brain metabolism (Wortis and Goldfarb^b) we have undertaken to establish the metabolic response of the brain to small doses of glucose administered intravenously to schizophrenic

^{*} Aided by a grant from the Havelock Ellis Fund for Psychiatric Research.

¹ Warburg, O., Posener, K., and Negelein, E., Biochem. Z., 1924, 152, 309.

² Himwich, H. E., and Nahum, L. H., Am. J. Physiol., 1930, 90, 389.

³ Lennox, W. G., Arch. Neurol. Psych., 1931, 26, 719.

⁴ Damashek, W., Myerson, A., and Stephenson, C., Arch. Neurol. and Psychiot., 1935, 33, 1.

⁵ Himwich, H. E., Bowman, K. M., Wortis, J., and Fazekas, J. F., J. Nervous and Mental Disease, 1939, 273.

⁶ Hoagland, H., Rubin, M. A., and Cameron, D. E., Am. J. Physiol., 1937, 120, 559.

⁷ Himwich, H. E., and Fazekas, J. F., Endocrinol., 1937, 21, 800.

⁸ Himwich, H. E., Frostig, J. P., Fazekas, J. F., and Hadidian, Z., Am. J. Psychiat., 1939, 96, 371.

⁹ Wortis, J., and Goldfarb, W., Science, 1940, 91, 270.

subjects during therapeutic insulin shock, to serve as a basis of comparison for the availability of other substrates for brain oxidations.

Experimental. The cerebral metabolism was estimated from the arterio-venous differences of O₂, CO₂, and glucose. The arterial blood was obtained from either the brachial or femoral arteries, and the venous blood was sampled from the internal jugular vein using the technic described by Myerson, et al.¹⁰ The blood samples were analyzed for O₂, CO₂ (Van Slyke and Neill¹¹), and glucose (Folin and Wu¹²). The velocity of circulation in the peripheral vascular system was estimated with the method of Robb and Weiss.¹³ The metabolism of the brain was estimated after the patients were in hypoglycemic coma, and again at various intervals after the administration of 4 g of glucose intravenously. The latter dose was chosen after some preliminary trial as the minimum amount required to rouse the patients. At the end of the experiment the patients were given the usual large amount of glucose to terminate the treatment.

The summary of data is presented in Table I. In 15 patients the administration of 4 g of glucose intravenously was sufficient to promptly rouse the patients from coma. In 13 experiments the

TABLE I.

Effect of 4 Grams of Glucose Intravenously on Cerebral Metabolism of Schizophrenic Patients in Coma.

Exp. No.	Min. after glueose	Volume % oxygen			Glucose		0.
		Art.	Ven.	Diff.	Art.	Ven.	Circ. Time
1	10	19.4	15.8	3.6			11
2	5	19.8	13.2	6.6			
2 3	5	17.9	15.1	2.8	57	57	10
4	9	21.4	13.1	8.3			14
4 5	9 5	18.1	8.4	9.6	48	40	12
6	4	19.0	12.3	6.7			
7	4 5 5	21.8	14.1	7.7	56	43	15
8	5	20.5	15.9	4.6	69	68	10
9	5	19.2	14.4	4.8	47	43	10
10		22.4	15.9	6.5	53	47	10
11	6 5 5	20.9	16.1	4.8			
12	5	19.6	14.4	5.2			
13	4	17.1	13.1	4.0	56	62	16
14	4	19.0	16.0	3.0	23	30	
15	4	20.9	17.2	3.7	42	49	
Avg		19.8	14.3	5.46	50	49	12.
) cases in insulin	10.0		2.0			
eoma		19.6	16.7	2.9	23	21	11.

¹⁰ Myerson, A., Halloran, R. D., Hirsch, H. L., Arch. Neurol. and Psychiat., 1927, 17, 807.

¹¹ Van Slyke, D. D., and Neill, J. M., J. Biol. Chem., 1924, 51, 523.

¹² Folin, O., and Wu, H., J. Biol. Chem., 1920, 41, 367.

¹³ Robb, G. P., and Weiss, S., Am. Heart J., 1932-3, 8, 650.

oxygen uptake of the brain was increased significantly above the average of a large control series, the average changing from 2.9 volumes % to 5.46 volumes % after glucose administrations. During coma the arterial glucose averaged 23 mg % and the venous sugar 21 mg %. After glucose administration the values were 50 and 49 mg % respectively. The circulation time revealed no change.

The estimation of brain metabolism in these experiments was based on the differences between the concentrations of various substances in the arterial and venous blood. We are in agreement with other investigators that this method does not measure the total metabolism of the brain, and that the observations are affected by changes in blood flow; Abramson, et al.,11 have observed an increased blood flow in the extremities of patients receiving insulin therapy for schizophrenia, and the authors concluded that the evidence strongly suggested that there was an increased blood flow through the brain. They believe that the reduced oxygen uptake found in insulin hypoglycemic coma may be attributable to this possibly increased blood flow through the brain. Abramson's observations, however, in contrast to ours, were made less than 2 hours after insulin injection, preceding the onset of coma. Moreover, it is well known that blood flow through the brain may vary independently of the blood flow in the periphery, and is principally controlled by the arterial blood pressure. The latter gradually falls if the hypoglycemia is not associated with convulsive seizures. Direct observation of blood flow during insulin hypoglycemia in rabbits reveals no significant changes unless convulsions occur (Leibel and Hall¹⁵). In humans a gradual decrease in brain blood flow occurs during insulin hypoglycemia (Loman and Myerson¹⁶). Such a decrease in blood flow indicates that there is probably an even greater diminution of brain metabolism than the diminished arterio-venous oxygen differences recorded by Himwich, Bowman, Wortis and Fazekas17 would indicate. It will be noted that the arterio-venous difference for glucose did not return to normal values with the return to a normal oxygen difference. This apparent discrepancy can probably be explained on the assumption that the glucose administered is rapidly absorbed by the tissues, including the brain tissue, and that its subsequent utilization within the cell is not accompanied by any further removal of glucose from the

¹⁴ Abramson, D. I., Schesloven, N., Margofis, M. N., and Musky, I. A., Am. J. Physiol., 1939, 128, 124.

¹⁵ Leibel, B. S., and Hall, G. E., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 894.

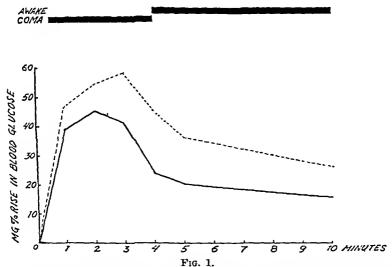
¹⁶ Loman, J., and Myerson, A., Am. J. Psychiat., 1936, 92, 791.

¹⁷ Wortis, J., Bowman, K. M., Goldf. 11b, W., Fazekas, J. F., and Himwich, H. E., .1m. J. Physiol., 1940, in press.

blood. Since there is no comparable intracellular storage of oxygen the increased oxygen uptake continues. This supposition is supported by reference to blood sugar curves after the administration of 4 g of glucose intravenously to patients in insulin coma. These show a rapid removal of glucose from the blood, preceding clinical arousal (Fig. 1) with a persistence of clinical recovery long after the blood glucose ceases to show any further considerable drop.

The rapid clinical arousal and the rapid increase in oxygen uptake, however, again confirm the ready availability of glucose as a metabolic substrate for the human brain, and affords a convenient yardstick for comparison with the availability of other foodstuffs for brain metabolism during hypoglycemic insulin coma.

Summary and Conclusions. The availability of glucose for brain oxidations in hypoglycemic insulin coma was studied in human patients. The intravenous administration of 4 g (in 50% solution) invariably aroused the patients and approximately doubled the oxygen uptake of the brain.



The solid line shows the amount of increase of blood glucose (antebrachial veiu) following intravenous administration of 4 g of glucose during insulin coma. The dotted line shows the amount of rise above fasting levels in the same patients on non-treatment days. The comatose patients almost invariably roused in about 4 minutes. The curves are based on averages of 13 experiments in coma, and 7 experiments outside of coma.

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¹⁴ Abramson, D. I., Schesloven, N., Margolis, M. N., and Mirsky, I. A., Am. J. Physiol., 1939, 128, 124.

¹⁵ Leibel, B. S., and Hall, G. E., Proc. Soc. Exp. Biol. and Med., 1938, 38, 894.

¹⁶ Loman, J., and Myerson, A., Am. J. Psychiat., 1936, 92, 791.

¹⁷ Wortis, J., Bowman, K. M., Goldfarb, W., Fazekas, J. F., and Himwich, H. E., Am. J. Physiol., 1940, in press.

nicotinuric acid diphosphopyridine nucleotide vitamin B6 hydrochloride dinicotinic acid alpha picoline alpha picoline methiodide beta picoline 5,6-dichlor-nicotinic acid trigonelline 5-amino-nicotinic acid 3,5-diamino-2,6-dimethyl pyridine 2,6-dimethyl pyridine 2,6-dimethyl pyridine hydrochloride 2.6-dimethyl dinicotinic acid (K salt) diurethyl lutidine (3,5-diurethyl-2,6-dimethyl pyridine) pyridine 2,3,5,6-tetracarboxylic acid 2,4,6-trimethyl dinicotinic acid (K salt) 1,4-dihydro-, 3,5-dicarbethoxy-, 2,4,6-trimethyl pyridine 1,4 dihydro-, 3,5-dicarbethoxy-, 2,6-dimethyl pyridine 3,5-dicarbethoxy-, 2,6-dimethyl pyridine 3,5-dicarbethoxy-, 2,4,6-trimethyl pyridine quinoxaline 2-3-dicarboxylic acid pyrazine monocarboxylic acid pyrazine 2,3-dicarboxylic acid 2-methyl, 3-hydroxy-quinoxaline

Most of these compounds gave negative results. Both compounds containing a 5-amino substitution in the pyridine ring gave a fluorescence with an indigo blue tint resembling that of thiochrome, rather than that of the unknown; alkali was not needed to bring this out. Both compounds containing the 1,4 dihydro-, 3,5 dicarbethoxy substitutions gave a strong fluorescence, the color in both instances showing distinct differences from the unknown-the dimethyl compound giving a deep blue fluorescence and the trimethyl a violet emission; fluorescence appeared without the addition of alkali. The 3,5 dicarbethoxy dimethyl and trimethyl pyridines gave, also without alkali addition, a bluish fluorescence somewhat resembling the unknown, but the spectra given by these solutions were not identical with that of the fluorescent urinary extracts, from which it would appear that these compounds were not identical with the unknown. Diphosphopyridine nucleotide showed a blue fluorescence similar to that of the unknown, which likewise developed only after the addition of alkali. The fluorescent spectrum, however, showed marked differences from the unknown. Since the fluorescent spectrum of diphosphopyridine nucleotide has not, so far as we are aware, been studied, it is reproduced herewith. (Fig. 1.)

None of the compounds tested could, therefore, be identified with

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Presence of a Hitherto Unrecognized Nicotinic Acid Derivative in Human Urine.*

VICTOR A. NAJJAR AND ROBERT W. WOOD. (Introduced by L. Emmett Holt, Jr.)

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Baltimore, Maryland.

In the course of some studies on the excretion of thiamin in urine by means of the thiochrome method, using the procedure of Hennessy and Cerecedo,1 it was noted that treatment of the KCl eluate of urine with alkali, even in the absence of ferricyanide, yielded a small amount of a substance soluble in butyl alcohol which gave a bluish fluorescence with ultraviolet light. This fluorescence could be distinguished from that given by thiochrome even with the naked eye, being a whitish blue without any tinge of violet. Specimens of urine from a large series of normal individuals of various ages were found to exhibit such fluorescence in slight degree. A patient receiving nicotinic acid therapy, however, was found to excrete it in large amount, suggesting that nicotinic acid was the precursor of this substance. Following this observation the effect of taking nicotinic acid was studied in normal individuals, and it was found that a dose of 50 mg of nicotinic acid, given to an adult, produced a prompt increase in the excretion of the unknown material. An increase in its concentration in the urine could be detected within an hour, and persisted for 4 to 6 hours.

We have attempted to identify the unknown urinary constituent by studies of the fluorescence of 27 different pyridine derivatives.† These compounds were dissolved in 25% KCl solution, both with and without treatment with alkali; the aqueous solution was then extracted with butyl alcohol, the alcoholic extract being tested for fluorescence with ultraviolet light. The following substances were tested:

nicotinic acid nicotinic acid amide

^{*} This study was aided by a grant from Mcad Johnson and Co., Evansville, Ind. 1 Hennessy and Cerecedo, J. Am. Chem. Soc., 1939, 61, 179.

[†] Most of these compounds were furnished us through the courtesy of Dr. Charles E. Bills. Dr. William A. Perlzweig was kind enough to supply us with samples of trigonelline and nicotinuric acid, and Dr. Eric G. Ball with the sample of diphosphopyridine nucleotide; the vitamin B₀ hydrochloride was furnished by Merck and Company.

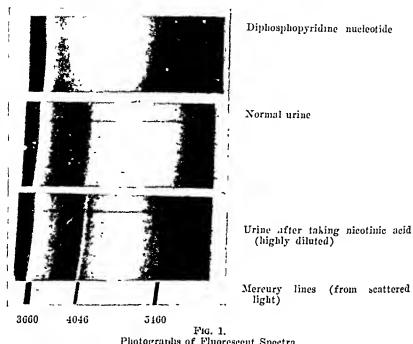
of normal urine and urine after nicotinic acid administration, the eluates being treated as before with alkali and extracted with butyl alcohol. The fluorescent spectra obtained, which are reproduced herewith, indicate that the substance present in small amount in normal urine is identical with that obtained in larger quantity after the ingestion of nicotinic acid. The photographs were taken with a small quartz spectrograph. The mercury lines appear faintly superimposed on the spectrum as the result of Rayleigh scattering of the solvent.

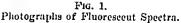
A number of observations were made upon the stability and differential solubility of the unknown substance. Boiling destroys the substance, more rapidly in the presence of alkali. At room temperatures it is destroyed slowly in alkaline solution and slowly by K₃Fe (CN)₀. The fluorescence of the butyl alcohol extract is readily destroyed by exposure to sunlight, but not by ultraviolet light passed through a Wood filter. It is readily extracted from aqueous solution by butyl or isobutyl alcohol, but not by amyl alcohol, octyl alcohol, chlorbenzene, benzene or chloroform.

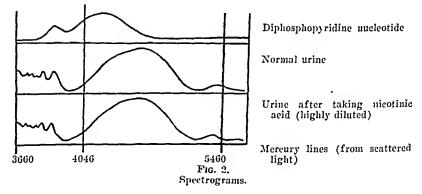
The probability that this substance represents an excretion product of nicotinic acid suggests that its measurement may prove of value in states of nicotinic acid deficiency in man; one might anticipate finding a low value with subnormal excretion following a test dose of nicotinic acid. Up to the present we have had no opportunity of testing patients with nicotinic acid deficiency. Through the courtesy of Dr. C. A. Elvehjem we have tested the urine of a dog with black tongue before and after treatment. We were, however, unable to identify the unknown fluorescent material in the urine of this animal either before or after treatment with nicotinic acid, which suggests that the dog may conjugate this substance by a different mechanism from that which occurs in man.

In the hope that others may have an opportunity to apply this procedure to pellagrins, it is given in detail:

To 20 cc urine are added 10 g permutit ("Decalso" 30 mesh) in a small separatory funnel; this is shaken for 15 minutes gently. The permutit is then washed with 5 portions (30 cc each) of distilled water, the washings being discarded. The permutit is then dried by suction. Ten cc of a 25% KC1 solution are then added and the funnel is shaken well for 15 minutes. The fluid is then allowed to drain from the permutit drop by drop the last portion being expelled by air. The permutit is washed with 2 to 4 cc of KC1 solution which is added to the cluate previously collected. The cluate is then divided into two equal portions, to one of which one







the unknown. An attempt was made to produce the unknown substance from nicotinic acid and from nicotinic acid amide in vitro by incubating normal urine with these compounds for 24 hours at 37° C. This procedure failed to increase the content of the unknown fluor-The possibility that the unknown material might escent material. be a porphyrin was considered; however, spectroscopic examination of the KC1 eluate failed to confirm this.

Photographs of the fluorescent spectrum were made with eluates

and we have employed it, with slight modifications of the original method, since 1937.

A mixture consisting of 7 volumes of 95% alcohol and 3 volumes of redistilled absolute ether is cooled to -20°C or -18°C. To this mixture 1 volume of serum, previously cooled to 4°C, is added drop by drop and very slowly, the contents of the container being vigorously stirred for the entire time. After all the serum has been added, the flask is well shaken and allowed to stand for 2 hours.

The resulting fine white precipitate of serum proteins is filtered off on a Büchner funnel and is washed repeatedly with absolute ether cooled to -20°C. Since as much alcohol as possible should be removed, it is desirable, whenever practical, to use 20 volumes of absolute ether for the washings at this stage. The filter paper with the protein is placed over sulphuric acid in a vacuum desiccator, which is then evacuated to remove the remaining ether. Solid carbon dioxide is used to cool the precipitating and washing fluids.

When this procedure was carried out with small amounts of serum (10-20 cc), the protein preparation obtained was in the form of a coarse white powder, which was slowly soluble. A solution of this preparation was perfectly clear and resembled the original serum in appearance. Clear solutions can still be obtained from the dried preparations made 2 years ago.

Three experiments were carried out to test the physiologic properties of dried proteins prepared by this method from dog serum. (1.) Ten gram portions of dried serum were dissolved in physiologic saline solution by prolonged stirring, after which the solution was centrifugalized for 30 minutes at about 3,000 rpm to remove any undissolved protein present. It was then filtered through a Seitz bacteriological filter and the protein content was checked by the colorimetric method of Johnston and Gibson.3 Twenty cc of the solution (2 g of protein) were injected intravenously into a dog after a preliminary temperature observation had been recorded. No alterations in temperature were observed in observations made at 15-minute intervals for several hours after the injection. (2.) The experiment was repeated with a different protein solution on another dog without ill effects. (3.) A third dog was given 50 cc of solution (5 g of protein) representing a combination of 2 other protein preparations. A transitory temperature elevation of 1°C was observed.

Experiments were then carried out with human serum proteins of appropriate blood type, which were dried, dissolved, Seitz-filtered,

³ Johnston, G. W., and Gibson, R. B., Am. J. Chn. Path. Tech. Suppl., 1938, 8, 22.

cc of 15% NaOH is added. Both samples are then shaken immediately with 13 cc butyl alcohol for 3 minutes. The mixture is then centrifuged to separate the butyl alcohol layer and this is treated with anhydrous Na2SO4 to remove traces of water, and is allowed to stand in the dark for 20 minutes. Fluorescence is then determined in a Pfaltz and Bauer fluophotometer, the source of light for which is a mercury vapor bulb (General Electric, type H3-85 watts) shielded by a Wood filter (Jena UG-2), the emitted fluorescence being measured after the interposition of a double filter of bright bluish green (Jena BG-14) and bright yellow (Jena GG-3). The difference in fluorescence between the sample treated with alkali and that not so treated represents the fluorescence of the unknown compound. Comparative quantitative measurements can be obtained by comparing the fluorescence with that of a quinine sulfate solution containing 10 to 25 µg % in 0.1 normal H2SO4. The daily output of the unknown substance in urine of normal adults gives a fluorescence corresponding roughly to 100 µg of quinine sulfate. After the ingestion of 50 mg of nicotinic acid, the concentration in urine reaches 8 to 10 times its previous value, during the first 4 hours.

11464 P

A Simple Method of Preparing Dried Serum Proteins for Therapeutic Use.

W. KNOWLTON HALL, DAVID E. FADER AND GEORGE M. DECHERD From the Departments of Biochemistry and Medicine, School of Medicine, Louisiana State University, New Orleans.

Attempts to preserve serum proteins in a dry form for therapeutic use have been made for a number of years. Flosdorf and Mudd's¹ method of preparing "lyophile" serum is for the most part satisfactory, but requires complicated and expensive special apparatus which was not at our disposal when we began to work with dried serum proteins. We therefore found it necessary to find or devise some method which could be carried out with ordinary laboratory apparatus. After preliminary trials with several other methods, we found the method devised by Hartley² for the preparation of dry and lipid-free immune sera to be most suitable,

¹ Flosdorf, E. W., and Mudd, S., J. Immunol., 1935, 29, 389.

² Hartley, P., Brit. J. Exp. Path., 1925, 6, 181.

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² Hartley, P., Brit. J. Exp. Path., 1925, 6, 181.

On the other hand, Heckel and Hori¹ found no noteworthy effects on spermatogenesis in 11 normal patients given sulfanilamide. Levaditi and Vaisman³, as well as Palazzoli, Nitti, Bonet, and Levinson, observed little effect from sulfanilamide on spermatogenesis in experiments with mice, rats and rabbits.

Since all of the work referred to was carried out in vivo, and as far as the author is aware, no experiments have been made on the in vitro effect of sulfanilamide on spermatozoa, the following studies were conducted on the effect of sulfanilamide and sulfapyridine as well.

Materials and Methods: The seminal specimens studied in these experiments were of varying freshness. They were furnished by 20 donors ranging from 17 to 51 years. Approximately 0.01 ml of semen was thoroughly mixed with 1 ml of Baker's fluid containing either sulfanilamide or sulfapyridine in concentrations of 5 to 160 mg %; the specimens thus mixed were kept both at 22°C (approximately) and body temperature. Control tests were conducted with the same amount of semen in Baker's fluid alone, also kept at the 2 temperatures. Sulfanilamide and sulfapyridine were also added to 1 ml of undiluted semen in a number of experiments, in an amount sufficient to give maximum concentration at the respective temperatures, undiluted semen alone being used as a control. Observations were made at frequent intervals, particularly toward the end of an experiment, so that the time of cessation of all movement could be accurately ascertained. Depression slides were used and while observations were not being made, they were kept in closed petri dishes, to prevent evaporation. The results obtained are presented in Tables I, II.

Results: The tables demonstrate no correlation between the age of the donor and the length of survival of the spermatozoa. There was marked individual variation in the duration of motility among different specimens, but the average time of survival at room temperature (9.4 hours) was considerably longer than at body temperature (3.5 hours). It can also be seen that the survival time of spermatozoa in various concentrations of sulfanilamide and sul-

⁴ Heckel, N. J., and Hori, C. G., Am. J. Med. Sci., 1939, 198, 347.

⁵ Levaditi, C., and Vaisman, A., Compt. rend. Soc. de biol., 1938, 128, 352.
6 Palazzoli, M., Nitti, F., Ronat, D., and Lovinson, M., Count. rend. Soc.

⁶ Palazzoli, M., Nitti, F., Bouet, D., and Levinson, M., Compt. rend. Soc. de biol., 1938, 128, 261.

⁷ Baker, J. R., J. Hyg., 1931, 31, 309.

t The composition of Baker's fluid is as follows: water, 1000 ml; glucose, 30.9 g; Na₂HPO₄ · 12 H₂O₅ 6.0 g; NaCl, 2.0 g; KH₂PO₄, 0.1 g.

tested for sterility, and injected into a dog. When no reaction was observed in the animal, 40 cc of the same solution (4 g of protein) was injected intravenously into a patient. There was no apparent reaction.

When serum was used in 500-700 cc portions, some difficulty was encountered in the preparation of dried serum proteins due to denaturation of the proteins. The cause of the denaturation is probably the difficulty of stirring the larger amount of precipitating fluid adequately, as well as difficulties in filtration. If filtration is not accomplished very rapidly, the alcohol in the precipitating fluid tends to become warm enough to denature the serum proteins. These difficulties have not yet been entirely overcome.

Summary. The method devised by Hartley for the preparation of dry and lipid-free immune sera has been adapted to the preparation of dried scrum proteins in quantity. In spite of some technical difficulties, the scrum proteins prepared by this method have been injected into dogs and into a single human subject without serious reactions.

11465

Resistance of Human Spermatozoa in vitro to Sulfanilamide and Sulfapyridine.

LANDRUM B. SHETTLES* (Introduced by N. J. Eastman)

From the Department of Obstetrics, The Johns Hopkins University and Hospital.

Jaubert and Motz² studied the effect of sulfanilamide on spermatogenesis in 23 men suffering from gonorrhea. They noted a reduction in both the number and vitality of the spermatozoa, with their complete immobilization in some instances. Marion, Barbellion, and Torres,² observed that small oral doses of sulfanilamide caused a decrease in the number and motility, and an increase in the abnormal forms, in the spermatozoa of 69% of their patients. Vigoni³ found the same changes in men treated by urethral irrigation with sulfanilamide, 2 of his patients actually developing azoospermia.

^{*} Fellow, National Committee on Maternal Health.

¹ Jaubert, A., and Motz, C., Presse méd., 1938, 46, 237.

² Marion, Barbellion and Torres, Bull. Soc. fran. d'urol., May 16, 1938.

a Vigoni, M., J. Belge d'urol., 1938, 11, 375.

fapyridine at both room and body temperatures was essentially the same as that of the respective controls. Neither the age of the specimen nor donor altered either susceptibility or resistance to these drugs. The results were equally negative if the sulfanilamide and sulfapyridine were added to Baker's solution plus semen or to undiluted semen.

Since an *in vitro* concentration of as much as 160 mg % was used, these results gain added significance when it is recalled that the maximum tissue fluid concentration of sulfanilamide achieved clinically is about 15 mg % and that of sulfapyridine about 10 mg %.

Summary: In vitro concentrations of sulfanilamide and sulfapyridine well above the tissue concentration achieved by therapeutic doses, do not affect the survival or activity of human spermatozoa.

11466

A New Salmonella Type Isolated from Apparently Normal Hogs.*

P. R. EDWARDS, D. W. BRUNER AND H. L. RUBIN

From the Department of Animal Pathology, Kentucky Agricultural Experiment Station, and Department of Bacteriology, University of Kentucky, Lexington, Ky.

In a study of the mesenteric lymph glands of apparently normal hogs in Uruguay, Hormaeche and Salsamendi¹ isolated numerous Salmonella types In a repetition of this work Rubin² found that Salmonella strains could be isolated frequently from the mesenteric lymph glands of apparently normal hogs slaughtered at an abattoir in Kentucky The purpose of the present paper is to describe a hitherto unrecognized Salmonella type encountered in these hogs. The organism is designated as Salmonella lexington.

Methods—Two mesenteric lymph glands were removed from each hog after the internal organs had been inspected. Lymph glands from 25 hogs were placed in a sterile container, taken to the labora-

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¹ Hormaeche, E, and Salsamendt, R., Arch Grug. Med., Cir. y Espec. 1939, 14, 375.

² Rubin, H. L., unpublished data.

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TABLE I.	Sulfanilamide a
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	Resistance

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tapyridius in Fitro at Room Temperature	Conc. of sulfapyridine, mg%	5 10 20 40 80 160 Survival time, hr	3.0 3.0 15.5 14.7 14.3 17.0 15.0 15.5	13.0 4.0 3.5 7.6 6.5 7.0 7.0 7.2 8.0	7.0 6.3	Cone. of sulfapyridine, mg%	urvival time, hr	2.0 6.0 4.0 3.1	4.7	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Cone. of suffanilamide unce.	Conc. of sulfanilamide, mg%	5 10 20 40 80 160 Survival time, hr	13.0 14.0 13.5 14.5 3.2 3.0 3.3 16.0 16.0 16.5	8.0 8.0 8.5 7.0 6.5	ermatozo	Conc. of Sulfanilanido, mg% 5 10 20 40 80 160	drvival time, hr	5.5 5.3 1.0 5.5 5.0 5.6 3.7 3.7 4.0 3.5 3.5 3.5	6.2	1.0 1.0 1.2 3.0 3.2 0.5 0.5 0.5 0.5
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Methods—Two mesenteric lymph glands were removed from each hog after the internal organs had been inspected. Lymph glands from 25 hogs were placed in a sterile container, taken to the labora-

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¹ Hormaeche, E., and Salsamendi, R., Arch. Urug. Med., Cir. y Espec., 1939, 14, 375.

² Rubin, H. L., unpublished data.

tory and thoroughly ground with sand in a sterile mortar. To the ground mass was added 30 cc of sterile saline and 1 cc of the mixture was placed in each of 3 tubes of the tetrathionate enrichment medium of Kauffmann.³ After overnight incubation the enrichment medium was plated on brilliant green agar. Salmouella-like colonies which developed on the plates were examined serologically and their position in the Kauffmann-White classification determined. The methods used in the serological tests were those employed by Edwards.⁴

Results—In the particular lot of glands from which the new type was isolated 3 Salmonella species were found. In addition to S. lexington, S. derby and S. bareilly were also present.

The microörganism was a motile rod which possessed the usual biochemical and tinctoral properties attributed to the genus Salmonella. It produced acid and gas from arabinose, dulcitol, glucose, inositol, mannitol, rhamnose, sorbitol, trehalose and xylose. Adonitol, lactose, salicin and sucrose were not attacked. Hydrogen sulfide was produced from 2% peptone water.

Examination of the somatic antigens of *S. lexington* revealed that it belonged to group E of the Kauffmann-White classification. Alcoholized suspensions were agglutinated to the titre of *S. nyborg* antiserum and absorption of the serum with *S. lexington* removed all somatic agglutinins for the homologous strain. The somatic antigens of *S. lexington* are III X XXVI

When the flagellar antigens of *S. lexington* were examined it was found that the organism was diphasic and displayed specific-non-specific phase variation. The specific phase was flocculated to the titre of serum derived from the alpha phase of *S. glostrup*, but was not affected by serums derived from the other antigens represented in the Kauffmann-White classification. Absorption of *S. glostrup* alpha serum with the specific phase of *S. lexingtou* removed all flocculating agglutinins for the homologous strain. The antigen of the specific phase of the organism is z_{10} .

The nonspecific phase of *S. lexington* was agglutinated by serums derived from all the nonspecific phases of the Kauffmann-White schema. It was then tested with absorbed serums containing the factors 2, 3, 5, 6 and 7. respectively. Agglutination occurred only in the presence of factors 3 and 5. When antiserum derived from the nonspecific phase of *S. choleraesnis* was absorbed with the nonspecific phase of *S. lexington* residual agglutinins amounting to less than

³ Kauffmann, F., Z. f. Hyg., 1935, 117, 26.

⁴ Edwards, P. R., J. Bact., 1936, 32, 259.

2% of the original titre were left for the homologous strain. The nonspecific antigens of S. lexington are 1,5...

Summary: A new Salmonella type, Salmonella lexington, is described. It was isolated from the mesenteric lymph glands of apparently normal hogs. The organism is represented by the antigenic formula III X XXVI:z₁₀:1,5...

11467 P

Mineral Distribution in Some Nerve Cells and Fibers.*

GORDON H. SCOTT

From the Department of Anatomy, Washington University School of Medicine, St. Louis.

It is known from examination of many types of tissue that Mg and Ca, as revealed by the electron microscope, is located in areas which show white ash following microincineration. In nerve tissue certain difficulties have hampered a direct study of Ca and Mg by means of the electron microscope. Some of the findings in incinerated sections of frog sciatic and sympathetic ganglia are believed to be of significance although we have not been able to identify the salts as clearly as is desirable.

When sections of frozen and dehydrated (Scott and Packer¹) frog sciatic are carefully incinerated and examined by dark field (Scott²) the large myelinated fibers at the periphery of the nerve leave residues of white ash probably consisting largely of Ca and Mg. The ash is clearly the remains of the myelin sheath as it corresponds almost exactly with stained preparations of the same nerve taken a few levels either above or below. The point to emphasize, however, is that there is no visible residue of any sort in the tissue spaces surrounding the nerve fibers.

In sharp contrast to plentiful mineral in the nerve fibers and little if any in the tissue space is the picture obtained when sympathetic ganglia are incinerated following the same treatment. The sympathetic ganglion cells are recognizable by their residue. Nuclear, nucleolar and Nissl substance ash is dense and of the variety associated with the presence of Ca and Mg. There is as a general rule

^{*} Aided by a grant from the Rockefeller Foundation.

¹ Scott and Packer, Anat. Rec., 1939, 74, 17, 31.

² Scott, G. H., Am. J. Anat., 1933, 53, 243.

a wide band, varying from an eighth to a sixth of the cell diameter, of dense white ash concentrated at the periphery of the cell. The tissue spaces immediately about the ganglion cells are filled with mineral residue not unlike, in quality and quantity, that seen in the neurones.

It seems evident from these observations that like conditions of surrounding medium do not obtain in nerve fibers of the frog sciatic and in the cells of the sympathetic ganglia of the same animal.

11468

Direct Action of Estrone on the Mammary Gland.

WM. R. LYONS AND Y. SAKO

From the Division of Anatomy, University of California Medical School.

Within the past decade, the hormonal control of mammary growth has been greatly clarified due to the availability of: (1) pure estrogenic compounds which by themselves cause growth of the nipple and ducts as well as a slight degree of alveolar development, (2) progesterone which in proper combination with estrone (or other estrins) causes complete lobular development such as occurs in pregnancy, and (3) mammotropin, the pituitary lactogenic hormone which causes functional growth and lactation in the alveoli or milk-secreting units in glands developed by estrin or estrin-progestin.

According to some investigators, the mammary glands of hypophysectomized animals do not respond as well (or at all) to estrin, and for this reason, Turner and co-workers^{1, 2} have proposed that the sex hormones merely stimulate the pituitary which in turn secretes 2 mammary-stimulating substances, mammogen I which induces duct development and mammogen II causing lobule-alveolar growth. These investigators have extracted a fat-soluble substance from the pituitary, which they identify with mammogen I because it causes mammary duct development in male and female mice. They³ also obtained lobule-alveolar growth in castrated female mice by injecting fresh pituitaries from pregnant cattle. Because this material caused alveolar proliferation over and above the duct development induced by their extracts these investigators have postulated a second mammogen.

¹ Lewis, A. A., Turner, C. W., and Gomez, E. T., Endocrinol., 1939, 24, 157.

² Lewis, A. A., and Turner, C. W., Mo. Agr. Exp. Sta. Bul. 182, 1939.

³ Mixner, J. P., Lewis, A. A., and Turner, C. W., Anat. Rec., 1940, Suppl., 76, 43.

The findings of the Turner laboratory might be interpreted on the basis of earlier investigations^{4, 5} showing that estrin and progestin could be extracted from the pituitary, but it has been claimed that mammogen I, although weakly estrogenic, has mammarystimulating potency greater than the purified estrins now available.2 While these findings are in themselves interesting and significant, they in no way support the contention of the Missouri group that the ovarian hormones act only indirectly upon the mammary gland. In fact, suggestive evidence to the contrary has been at hand for some time now.6 In each of 3 women, MacBryde obtained good development in the breast rubbed with estradiol in ointment (25,000 I.U., daily for 2 weeks) while the contra-lateral breast rubbed only with the ointment base showed much less growth. We have carried out in male rabbits a similar experiment designed to sustain the theory of the direct action of estrin, with the advantage over the human work reported, of being able to know more precisely the histologic nature of the growth response.

Experimental. Preliminary experiments were carried out in which the approximate amount of estrin was determined that could be expected to act only on the gland locally treated, and also the amounts of hormone that would be sufficient to allow for some absorption and therefore remote action on the glands not treated directly. And then, 2 groups of three, 2-month-old male rabbits, weighing approximately 2 kg were treated with 2 distinct levels representing threshold and sub-threshold doses of estrone.

The estrone* in sesame oil was rubbed into the skin immediately around the nipples on the left side, while sesame oil alone was administered in like manner to the nipple regions on the right side. Three of the rabbits had an accessory or ninth nipple and this was left untouched, thus serving as a further control. A single drop of oil (approximately 0.03 cc) was applied with a medicine dropper directly to the nipple. The oil usually spread out over a skin area not greater than 3.0 cm and this was rubbed with the end of the finger and gently massaged between the thumb and forefinger. The 3 rabbits in Group 1 were treated with an estrone preparation containing 100 I. U. per cc or 3 I.U. per drop, while the preparation used in Group 2 contained 10 I.U. per cc or 0.3 I.U. per drop. In all 25 drops were administered to each gland (Monday through

⁴ Broulia, L., and Simmonet, H., C. R. Soc. de Biol., 1927, 96, 1275.

⁵ Callow, R. K., and Parkes, A. S., J. Physiol., 1936, 87, 28 P.

⁶ MacBryde, C. M., J. A. M. A., 1937, 112, 1043 (has earlier references).

^{*} Kindly supplied by Dr. Oliver Kamm of Parke, Davis and Company.

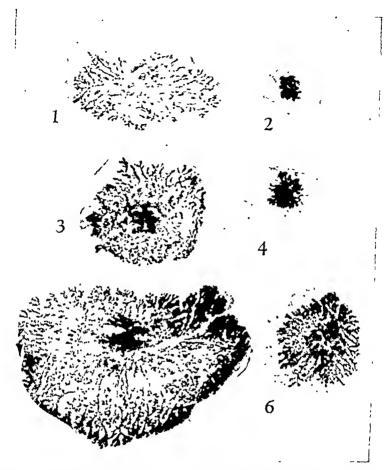


Fig. 1. Typical left mammary spread from male rabbit 1 of group 2. A drop of sesame oil containing approximately 3 I.U. of estrone was rubbed into the skin overlying this gland daily for 25 days. Note evidence of extensive duet growth. All figures \times 1.5.

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Fig. 2. Typical right mammary spread from male rabbit of group 2. Sesame oil only was rubbed into skin. Gland is rudimentary and resembles that of untreated, normal male rabbits.

Fig. 3. Typical left mammary spread from male rabbit 2 of group 2. A drop of sesame oil containing approximately 3 I.U. of estrone was rubbed into the skin overlying this gland daily for 25 days. Note evidence of extensive duct growth.

Fig. 4. Typical right mammary spread from male rabbit 2 of group 2. Sesame oil only was rubbed into skin. Gland is rudimentary and resembles that of nu-

treated, normal male rabbits.

Fig. 5. Typical left mammary spread from male rabbit 3 of group 2. A drop of sesame oil containing approximately 3 I.U. of estrone was rubbed into the skin overlying this gland daily for 25 days. This gland shows more extensive duct growth as well as some alveolar development.

Fig. 6. Typical right mammary spread from rabbit 3 of group 2. Although only sesame oil was rubbed into skin overlying this gland, enough estrone was absorbed on the left side to circulate and cause the duct growth shown here.

Friday for 5 weeks), making a total of approximately 75 I.U. per gland or 300 I.U. per animal (4 glands) in the first group, and 7.5 I.U. per gland or 30 I.U. per rabbit in the second group.

Results. It became apparent after 2 to 3 weeks of treatment that the nipples receiving the 3 L.U. estrone per dose were growing at a faster rate than the controls as well as those receiving the 0.3 L.U. estrone. At the end of the fifth week, the estrone-treated nipples of both groups were all considerably larger than their contra-lateral controls, but the control nipples of Group 1 were larger than the control nipples of Group 2 indicating that some of the estrone was being absorbed and probably circulated to the contra-lateral glands. At necropsy spreads were made of 53 mammary glands. These were stained in toto with alum carmine and cleared in methyl salicylate. None of the glands taken from the animals in Group 1 showed development greater than that seen in normal males of the New Zealand White strain used. Two of the 3 rabbits treated with higher doses of estrone showed duct growth only in the glands rubbed with the hormone, Figs. 1 and 3, the sesame control and untreated glands all being within the normal limits (Fig. 2 and 4). The third animal of this group was more responsive to estrone for not only did its estrone-treated glands (Fig. 5) show better development than the estrone-treated glands of the other rabbits, but its control glands (Fig. 6) also showed considerable development. As in the case of the nipples in both groups, this indicated that some estrone was being absorbed and circulated. However, the striking difference between the hormone-treated and control glands provides just as good proof of the direct action of estrone as the all-or-none effect observed in the other animals.

Ruinen⁷ had attempted this same experiment in 1932, but used a much larger dose of hormone (100 units daily of menformon). With such an excess, he obtained equal development in the mammary glands of both sides. As the author admits, his findings still did not preclude the possibility of a direct action of estrin on the mammary gland.

Summary. The proper dose of estrone in oil rubbed into the skin over the rudimentary mammary glands of young male rabbits caused growth only of those glands, and not of the control glands treated with oil. Such evidence supports the view that estrogenic substances are directly mammary-stimulating.

⁷ Ruinen, F. H., Acta Brev. Neerl., 1932, 2, 161.

11469 P

Maintenance of Pregnancy in Castrate Rats by Means of Progesterone.*

IRVING ROTHCHILD AND ROLAND K. MEYER
From the Department of Zoology, University of Wisconsin, Madison

Corner and Allen's early successful attempts to maintain pregnancy in rabbits castrated shortly after mating could not be duplicated by Allen and Heckel² using crystalline progesterone, unless the castration occurred after implantation.³ Pincus and Werthessen,⁴ however, maintained gestation in one out of three rabbits when sufficient crystalline progesterone was used, while in short time experiments Courrier and Kehl⁵ maintained pregnancy in rabbits with progesterone, but in only one rabbit was castration done before implantation. Courrier and Jost⁶ using large amounts of pregneninolone caused rabbits to implant normally. Maintenance of pregnancy has been successfully accomplished in other animals (rats,^{7, 8} ground squirrels,⁸ mice⁰ and hamsters¹⁰) using progestin,⁸ progesterone⁰, ¹⁰ or androgens,⁷ when castration was done after implantation.

Previously¹¹ it was shown that placentomata indistinguishable from those formed in normal pseudopregnant rats could be produced in the castrate rat by means of progesterone alone. In the present study it will be shown that normal implantation as well as continued gestation will occur in the castrate rat when sufficient progesterone is administered.

A total of 26 pregnant rats were used, 22 of which were castrated on the 4th, and 4 on the 10th day of pregnancy. All rats, except

^{*}This work was supported by the Wisconsin Alumni Research Foundation. Assistance was also furnished by the personnel of the WPA Official Project No 65 1 53-2349.

¹ Corner and Allen, Proc. Soc. Exp. Biol. and Mfd, 1930, 27, 403

² Allen and Heckel, Science, 1937, 86, 409.

³ Allen and Heckel, Am. J. Physiol , 1939, 125, 31.

i Pineus and Werthessen, Am. J. Physiol, 1938, 124, 484.

⁵ Courrier and Kehl, Comptes rendus Soc. de Biol , 1938, 128, 188.

⁶ Courrier and Jost, ibid , 1939, 130, 1162.

⁷ Greene and Burrill, Proc. Soc Exp. Biol. and Med , 1939, 42, 595.

⁸ Johnson and Challans, Endocrinol, 1932, 16, 278.

⁹ Robson, J. Physiol, 1938, 92, 371.

¹⁰ Klein, Proc. Roy. Soc, B, 1938, 125, 348.

¹¹ Rothchild, Meyer and Spielman, Am. J. Physiol, 1940, 128, 213

those in the first 2 experiments, were sterilized by ovario-salpingectomy of one horn of the uterus on the day after coitus, and the sterile horn traumatized by needle punctures through the antimesometrial wall on the 4th day of pregnancy. This permitted a concomitant study of the effect of the hormone treatment on the formation of placentomata. Hormone treatment was started on the day of complete castration, and continued daily up to and including the 20th day of pregnancy. All rats were autopsied on the 21st day, except in those cases where, by means of laparotomies performed between the 9th and 17th day, it was seen that pregnancy had terminated. Hormone treatment consisted of progesterone[†] alone, or in combination with estradiol, with corn oil as the solvent.

From Table 1 it can be seen that negative results were obtained with amounts of progesterone of less than 1 Rb.U. per day. The smaller doses of progesterone, however, permitted the formation of implantation sites, which in the rats of Exp. I did not persist beyond the 13th day, but in 2 of the 4 rats of Exp. II persisted normally to

TABLE	I.
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		aily e treatment		Ra	Rats maint. in pregn. to 21st day			
Exp. No.		Estradiol, y	Rats treated	No.	Implant. Sites	Term Fetuses		
I	.3	.03	4	0				
II	.6	.03	4	0				
III	1.0	-	5	2	5 5	1 1		
IV	1.0	.15	3	1	2	1		
Vt	2.0	_	4	3	4 3 4	3 2 4		
VI	2.0		5	4	2 3 4 4	1 2 3 1		

^{*} Rabbit units of progesterone.

These rats were eastrated on the 10th day of pregnancy.

t The source of progesterone was a non-crystalline preparation containing 10%-50% of progesterone (Corner-Allen Rabbit Units) and was made from cholesterol by the Spielman process. (Spielman and Meyer, J. A. C. S., 1939, 61, 893.)

 $[\]ddagger$ The estradiol was supplied through the courtesy of the Schering Corporation, Bloomfield, N. J.

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abnormalities in the state of the serum proteins might occur in this disease. Perlzweig, Kondritzer and Bruch³ found that in pathological conditions fractional precipitation of the serum proteins with gradually increasing quantities of potassium phosphate at pH 6.5-6.8, according to Butler and Montgomery,⁴ frequently reveals significant changes which are not brought out by the conventional methods for the determination of albumins and globulins by precipitation with an arbitrarily fixed concentration of a neutral salt.

In the present investigation the fractional precipitation procedure was applied to the serum proteins of 8 physically healthy patients in whom a diagnosis of phenylpyruvic oligophrenia had been made, and 8 healthy persons as controls. Nineteen portions of each sample of serum were precipitated with an equimolar KH₂PO₄-K₂HPO₄ buffer which ranged in molality from 1.2 to 3.0 mols in 0.1 mol increments. In 5 of the patients and 2 of the normal subjects 0.5 cc portions of freshly centrifuged serum were added to 15 cc portions of buffer (serum dilution 1:31). In the remaining experiments in which less serum was available the serum was diluted with an equal volume of physiological saline; then 0.5 cc portions were added to 10 cc portions of the buffer solutions (serum dilution 1:42). After standing overnight at room temperature the precipitate was filtered off and the total nitrogen in solution was determined (microkjeldahl) on a suitable aliquot. The value was corrected for the N.P.N., determined on a trichloracetic acid filtrate of the original serum, and the percentage of the total protein remaining in solution at each molality of phosphate was calculated. The data obtained in experiments in which the dilution of the serum was 1:42 were calculated to a dilution of 1:31 and combined with the data at the latter dilution. The average values obtained in the 8 normal and the 8 phenylpyruvic sera were plotted against the concentrations of the phosphate solutions (Fig. 1).

A small but consistent difference between the two solubility-precipitation curves in the middle range of phosphate concentration is apparent; more protein appears to have been precipitated from the sera of the phenylpyruvics than from those of the healthy controls. As the differences were small the data were subjected to rigorous statistical analysis with the kind assistance of Dr. Joseph Zubin. The methods which Fisher⁵ developed for analyzing small series of

³ Perlzweig, W. A., Kondritzer, A. A., and Bruch, E., Proc. Am. Soc. Biol. Chem., 1938, 32, xeii.

⁴ Butler, A. M., and Montgomery, H., J. Biol. Chem., 1932, 99, 173.

⁵ Fisher, R. A., Statistical Methods for Research Workers, 1934. Edinburgh and London.

the 17th day. Implantation proceeded normally, as far as could be determined macroscopically, in all the remaining experiments, and at least some of the rats in each experiment carried living young to the 21st day. The percentage of completely maintained rats, as well as the ratio of living young to total number of implantations, increased with increase in the amount of progesterone administered.

The placentomata which formed in the sterile horn of the uteri of all the rats of Exp. III-VI were larger in every case than the implantation sites in the pregnant horn. This would indicate that the formation of decidual tissue in the rat is dependent, not only upon the size of the progesterone dose, 11 but possibly upon the strength of the traumatic stimulus as well, since it is most likely that the trauma of the uterine epithelium produced by the implanting egg is not of the same order of magnitude as that used in the artificial production of placentomata.

The possibility that contaminants in the progesterone preparation might have influenced the results must be admitted, but we do not believe that they played an important part. In other experiments.^{11, 12} using the same type of preparations, we found no quantitative or qualitative differences between the non-crystalline and crystalline progesterones.

Summary. Rats castrated on the 4th day of pregnancy were maintained in pregnancy until the 21st day with daily doses of progesterone of 1 or 2 Rb.U.

11470 P

Precipitation Pattern of Serum Proteins in Phenylpyruvic Oligophrenia.

ALBERT A. KONDRITZER (Introduced by Warren M. Sperry)

From the Neuro-Psychiatric Institute of the Hartford Retreat, Hartford, Coun., and the Department of Biochemistry, New York State Psychiatric Institute and Hospital, New York City.

Jervis, et al., have shown that the serum of phenylpyruvic oligophrenic individuals contains an abnormal amount of phenylalanine. In view of the recognized effects of small amounts of amino acids on the molecular dispersion of the proteins it seemed possible that

¹² Rothchild and Meyer, Anat. Rec., 1939, 75, supl. 1, 71.

¹ Jervis, G. A., Block, R. J., Bolling, D., and Kanze, E., in press.

² Tiselius, A., Ann. Rev. Biochem., 1939, 8, 155.

tion was calculated from three nitrogen estimations (protein and N.P.N. in the original serum, and the total nitrogen of the filtrate). The chances for a cumulative error are, therefore, rather high. However, all determinations were carried out in the same apparatus under identical conditions, as nearly as they could be controlled, and hence the statistical treatment evaluated automatically the effect of errors arising in the analysis. Furthermore, errors in the determination of the protein and N.P.N. of the original serum would have a uniform effect throughout for each sample of serum and could not account for significant deviations in a portion of the curve.

It is concluded that there was a small but significant increase above normal in the globulin fraction of the serum proteins of the individuals with phenylpyruvic oligophrenia studied in this investigation. It is possible that the increase may have resulted from factors other than the particular pathology involved in this disease.

11471

Response of Various Breeds of Rabbits to Hamilton and Schwartz Test for Parathyroid Secretion.

EMIL J. BAUMANN AND DAVID B. SPRINSON
From the Laboratory Division, Montefore Hospital, New York.

In our earlier work¹ with the Hamilton and Schwartz² test for parathyroid hormone, we used 2 breeds of rabbits. raised by ourselves; a black and white Dutch strain and a gray Belgian strain, which included some albinos. All these rabbits gave a positive test when injected with 10 Hansen units of parathormone per kg. In a few instances injection with as little as 4 units per kg resulted in a positive reaction. Similar responses were given by hybrids of these 2 strains. (The H. and S. test depends upon the fact that successive feedings of CaCl₂ result in smaller and smaller rises in serum Ca, so that after the 3rd or 4th administration of 100 mg of Ca as CaCl₂ normal rabbits will show a rise of serum Ca of less than 1.2 mg per 100 cc, whereas if more parathyroid hormone than that normally circulating is present, a greater rise of serum Ca results, roughly proportional to the quantity of hormone administered.)

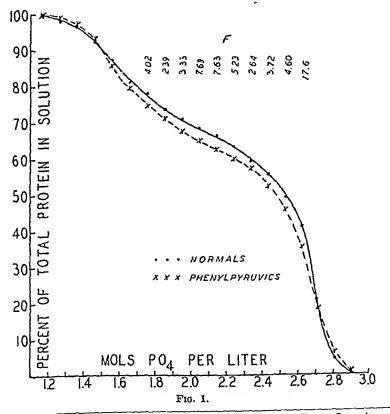
[†] The phenylpyruvic blood samples were obtained from immates of Letchworth Village through the courtesy of Dr. Harry C. Storrs, Superintendent.

¹ Baumann, E. J., and Sprinson, D. B., Am. J. Physiol., 1939, 125, 741.

² Hamilton, B., and Schwartz, C., J. Pharm. and Exp. Therap., 1932, 46, 285.

data were applied in the modification of Snedecor.6 The F value obtained gives a measure of the probability that the difference between 2 means is significant. For averages obtained on 2 series of 8 determinations each the critical values of F those which could arise by chance not more than 1 to 5 times in 100, lie between 9.07 and 4.67 respectively. It will be seen from the chart that F values in this range were obtained for 3 phosphate concentrations between 2.0 and 2.3 mols per liter.* The chance that fortuitous differences of this degree of significance would occur at adjacent points is exceedingly small.

Each value for the percentage of the protein remaining in solu-



⁶ Snedecor, G. W., Analysis of Variance, 1394, Collegiate Press, Inc., Ames,

^{*} For this range of phosphate concentrations the effect of the difference in dilution (1:31 and 1:42) on the solubility of the serum proteins was shown during the statistical analysis to be of no significance.

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When New Zealand white or chinchilla rabbits were used for this test, it was found they were less sensitive than the Dutch or Belgian breeds. They required an injection of 20 or 30 units of parathormone per kg to react positively. Six chinchillas and 4 New Zealand whites all gave negative reactions when injected with 10 units per kg. With a dose of 20 units per kg of parathyroid extract only 3 of 5 chinchillas and 1 of 3 New Zealand white rabbits reacted positively, while 2 of each of these strains gave positive reactions only with a dose of 30 units per kg.

It is advisable, therefore, to determine the sensitivity of rabbits to be used for the Hamilton and Schwartz test. The animals should be at least 5 months old and they should be kept on a diet whose Ca:P ratio is one or more for several days before use.

11472 P

Agent of Lymphogranuloma Venereum in the Lungs of Mice.

MORRIS F. SHAFFER, GEOFFREY RAKE AND CLARA M. McKee From the Squibb Institute for Medical Research, New Brunswick, N. J.

It has been shown¹ that the agent of lymphogramhoma venereum readily initiates a fatal infection when introduced into the yolk-sac of the developing chicken embryo, in contradistinction to the well known low-grade character of the infection which results when the virus is placed on the chorio-allantois. In the former site the minute "granulocorpuscles"² which are believed to represent elementary bodies of the agent are found in enormous numbers. With this source of abundant virus at hand the possibilities of intranasal infection in mice were investigated, as has also been done recently by Schoen³ who employed virus propagated in the Ehrlich mouse sarcoma.

Two strains* of the lymphogranuloma venereum agent were

¹ Rake, G., McKee, C. M., and Shaffer, M. F., Proc. Soc. Exp. Biol. and Med., 1940, 43, 332.

² Miyagawa, Y., Mitamura, T., Yaoi, H., Ishii, N., Nakajima, H., Okanishi, J., Watanabe, S., and Sato, K., Jap. J. Exp. Med., 1935, 13, 733.

³ Schoen, R., C. R. Acad. Sci., 1939, 208, 772.

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studied in Swiss mice weighing usually less than 10 g. Ground suspensions of yolk-sacs heavily infected with virus were diluted tenfold with broth and centrifuged to throw down tissue fragments. 0.03 to 0.05 ml of supernatant was inoculated intranasally under light ether anesthesia. The mice which within 48 to 72 hours were manifestly ill with signs of marked respiratory involvement, were sacrificed; some died during this period. The former at autopsy showed, in one or more lobes, areas of semi-translucent gray-red consolidation varying in extent; the dead mice showed hemorrhagic consolidation of nearly all of the lung tissue. On microscopic examination of smears made by streaking a fragment of consolidated lung on a slide, fixing the film in methyl alcohol and staining with Giemsa stain, numerous elementary bodies could be seen lying free or within monocytic cells. In histological sections the picture was one of pneumonia, varying in degree but often very intense. This was both interstitial, with an accumulation of fluid and cells in the walls of the alveoli, and lobular with filling of the alveoli with fluid, monocytes and neutrophils. In the cytoplasm of certain cells, apparently the lining cells of the alveoli, there could be seen in most lungs large vacuoles filled with elementary bodies or larger inclusions both of which stained purple with Giemsa. In addition, clumps of elementary bodies were seen lying free in the alveoli and bronchioles. The microscopic changes during the development of the lung lesions will be described in greater detail elsewhere.

Serial intranasal passages in mice under light ether anesthesia were readily effected by sub-inoculation of broth suspension of affected lung tissue taken on the 2nd to 4th days after infection. With one strain 39 such lung-passages have been attained; with the other strain, 24 passages. Mice given intranasally 10^{-2} dilution of grossly diseased lung tissue usually died within 5 days with almost total pneumonic consolidation. Fatal illness was less frequent in animals receiving 10^{-3} dilution; many of these mice exhibited a transient malaise but eventually recovered. If sacrificed between the 3rd and 5th days, however, macroscopic lung lesions were found in most mice receiving 10^{-3} dilution. Lesions were not found in mice receiving 10^{-4} and 10^{-5} dilutions. Nevertheless, at the end of 4 days' infection virus was present in considerable amount in the lungs of mice receiving 10^{-4} dilution as could be demonstrated on serial intranasal passage, and it seems certain that multiplication had occurred.

The possibility that the lesions obtained in the lungs were due to organisms of the pleuro-pneumonia group or to infection with the latent virus of mouse pneumonia was excluded, in the first case by

408 Lymphogranuloma Venereum in Mouse Lung

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Two strains* of the lymphogranuloma venereum agent were

¹ Rake, G., McKee, C. M., and Shaffer, M. F., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 332.

² Miyagawa, Y., Mitamura, T., Yaoi, H., Ishii, N., Nakajima, H., Okanishi, J., Watanabe, S., and Sato, K., Jap. J. Exp. Med., 1935, 13, 733.

³ Schoen, R., C. R. Acad. Sci., 1939, 208, 772.

^{*} One strain was obtained through the courtesy of Dr. Wm. L. Fleming, the School of Hygiene and Public Health, Johns Hopkins University. The second was obtained through the courtesy of Dr. Marion Howard, Department of Medicine, Yale University.

studied in Swiss mice weighing usually less than 10 g. Ground suspensions of yolk-sacs heavily infected with virus were diluted tenfold with broth and centrifuged to throw down tissue fragments. 0.03 to 0.05 ml of supernatant was inoculated intranasally under light ether anesthesia. The mice which within 48 to 72 hours were manifestly ill with signs of marked respiratory involvement, were sacrificed; some died during this period. The former at autopsy showed, in one or more lobes, areas of semi-translucent gray-red consolidation varying in extent; the dead mice showed hemorrhagic consolidation of nearly all of the lung tissue. On microscopic examination of smears made by streaking a fragment of consolidated lung on a slide, fixing the film in methyl alcohol and staining with Giemsa stain, numerous elementary bodies could be seen lying free or within monocytic cells. In histological sections the picture was one of pneumonia, varying in degree but often very intense. This was both interstitial, with an accumulation of fluid and cells in the walls of the alveoli, and lobular with filling of the alveoli with fluid, monocytes and neutrophils. In the cytoplasm of certain cells, apparently the lining cells of the alveoli, there could be seen in most lungs large vacuoles filled with elementary bodies or larger inclusions both of which stained purple with Giemsa. In addition, clumps of elementary bodies were seen lying free in the alveoli and bronchioles. The microscopic changes during the development of the lung lesions will be described in greater detail elsewhere.

Serial intranasal passages in mice under light ether anesthesia were readily effected by sub-inoculation of broth suspension of affected lung tissue taken on the 2nd to 4th days after infection. With one strain 39 such lung-passages have been attained; with the other strain, 24 passages. Mice given intranasally 10⁻² dilution of grossly diseased lung tissue usually died within 5 days with almost total pneumonic consolidation. Fatal illness was less frequent in animals receiving 10⁻³ dilution; many of these mice exhibited a transient malaise but eventually recovered. If sacrificed between the 3rd and 5th days, however, macroscopic lung lesions were found in most mice receiving 10⁻³ dilution. Lesions were not found in mice receiving 10⁻⁴ and 10⁻⁵ dilutions. Nevertheless, at the end of 4 days' infection virus was present in considerable amount in the lungs of mice receiving 10⁻⁴ dilution as could be demonstrated on serial intranasal passage, and it seems certain that multiplication had occurred.

The possibility that the lesions obtained in the lungs were due to organisms of the pleuro-pneumonia group or to infection with the latent virus of mouse pneumonia was excluded, in the first case by

cultures on suitable media and in the second, by cross-neutralization tests with anti-sera supplied through the courtesy of Dr. F. L. Horsfall.

In the case of both strains, inoculation of 1 ml of 10-7 and occasionally 10-s dilution of consolidated lung tissue into the yolk-sacs of 5- or 6-day eggs incubated at 36° C sufficed to bring about death of the developing chicken embryos after several days' infection. The yolk-sacs of these eggs examined immediately after death were bacteriologically sterile but showed innumerable elementary bodies in Giemsa-stained smears. These findings not only confirm the microscopic observations as to the presence of large amounts of virus in the lungs of mice intranasally infected with lymphogranuloma venereum agent but also serve to reemphasize the delicacy of the yolk-sac technic as compared with all other methods at present available for the detection of the virus. The obvious usefulness of the pulmonary infection t of mice in immunological experimentation on lymphogranuloma venereum is being further explored.

11473

Complement Fixation Test in Lymphogranuloma Venereum.

CLARA M. MCKEE, GEOFFREY RAKE AND MORRIS F. SHAFFER From the Squibb Institute for Medical Research, New Brunswick, N. J.

Although it is generally acknowledged that the cutaneous test with the Frei antigen is of great value in establishing the diagnosis of infection with the etiological agent of lymphogranuloma venereum, many workers have sought to devise other procedures which might be employed as corroborative evidence. The serological technic most widely explored in this connection has been complement fixation but, using a variety of antigens, most investigators have been unsuccessful in their attempts to demonstrate a specific reaction (for literature see1). Nearly all the reports of positive findings are justly open to criticism on such grounds as inadequately detailed description of the method, lack of controls, or incomplete data on the results.

⁴ Horsfall, F. L., and Hahn, R. G., J. Exp. Med., 1940, 71, 391.

t Workers should bear in mind the possible hazards involved in the use of the intranasal technic where high concentrations of virus are concerned. 1 Melezer, N., and Sipos, K., Arch. f. Dermat. u. Syph., 1937, 176, 176.

Since the quantitative relationships of the reagents are of paramount importance in serological tests it seemed likely that many of the failures might be due to the use of antigens of insufficient potency and that it might be worthwhile to reinvestigate the potentialities of the complement fixation test in this disease, with preparations containing higher concentrations of antigen than had hitherto been avail-The following antigens have been employed: (a) the consolidated lungs of several mice, infected intranasally2 with the agent of lymphogranuloma venereum, were pooled and ground with Pyrex fragments plus broth to a 10% suspension. This was freed of gross particles by centrifugation at 2000 RPM. The supernatant, termed "Lygranum" (M.L.) antigen, was stored at -32°C until needed, when it was thawed and further diluted with 0.85% saline to a final lung concentration of 1:100 or 1:150 for use in the test. Normal mouse lung suspension similarly prepared served as control. (b) Five-or 6-day eggs. inoculated via the yolk-sac³ with lymphogranuloma venereum agent, were incubated at 36° C until the death of the chicken embryo. Immediately thereafter the bacteriologically sterile yolk-saes, heavily infected with virus, were removed and ground with Pyrex fragments plus broth to 10% suspension. This was centrifuged I hour at 2000 RPM; the supernatant was recentrifuged in the cold for 2 hours at 12,000 RPM and the sediment obtained thereby was resuspended in saline to 10 times the volume of the original 10% yolk-sac suspension for use in the test. This was called "Lygranum" (Y.S.) antigen. The resuspended sediment from normal yolk-sacs, treated in the same way, was used as control.

The sera were inactivated at 56° C before use and dilutions were made in saline. The source of complement was pooled guinea-pig serum kept frozen at -32° C. Previous to each test the thawed complement was titrated and diluted in saline so that 2 hemolytic units were contained in 0.2 cc. In the test 0.2 cc of each reagent was added to the tubes in the following order: serum dilution, complement, and antigen. The well-shaken mixtures were placed for 1½ hours at 37° C. Then to each tube was added 0.2 cc of 3% suspension of washed sheep cells sensitized with 2 minimal hemolytic doses of anti-sheep cell rabbit amboceptor. Readings for hemolysis were made after a further period of 30 minutes at 37° C. Controls for free complement and for anticomplementary action in each antigen and serum were always included. The titre of a given serum

² Shaffer, M. F., Rake, G., and McKee, C. M., Proc. Soc. Exp. Biol. and Med., 1940, 44, 408.

³ Rake, G., McKee, C. M., and Shaffer, M. F., Proc. Soc. Exp. Biol. and Med., 1940, 43, 332.

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was taken as the highest dilution showing complete or nearly complete fixation with specific antigen and no fixation with the antigen control prepared from the corresponding normal tissues. The results with "Lygranum" (Y.S.) ran entirely parallel to those obtained with "Lygranum" (M.L.), although under the conditions of concentration employed in these experiments, the former antigen seemed more active. On the other hand, non-specific fixation in low serum dilutions with the antigen control from normal tissue was less frequently encountered in the case of mouse-lung. In performing the test the lowest initial serum dilution was 1:2 and since, in the system for fixation, this underwent a further threefold dilution, the lowest possible titre in these experiments was 1:6.

Using the method outlined, sera from 20 individuals with clinical nistory of lymphogranuloma venereum and positive reactions to Frei antigen were tested. Nineteen sera fixed complement specifically in the presence of "Lygranum" antigen, at titres ranging from 1:15 to 1:600. In one case the titer was only 1:6. The possible correlation between serum titre, degree of reactivity to standardized Frei antigen and the clinical status of lymphogranuloma infection in a group of cases is being investigated in collaboration with Dr. A. W. Grace of the New York Hospital, through whose courtesy most of the lymphogranuloma sera were obtained. As a control, sera taken from 22 presumably normal individuals were tested; 20 failed to fix complement even in the lowest dilution (titre less than 1:6). Positive reactions in this group were obtained only with serum from one laboratory worker who had been exposed to contact with the virus over the period of a year and with the serum of a female child residing in an orphanage.

As a further control, the test was carried out on a group of 29 sera with strongly positive Wassermann reaction obtained through the courtesy of Mr. J. V. Mulcahy of the State Dept. of Health, Trenton, N. J. and Dr. B. Webster of the New York Hospital. Sixteen of these sera gave specific fixation with "Lygranum" antigens showing titres between 1:15 and 1:150. In view of the reports concerning positive reactions to the Frei antigen, elicited in prostitutes who were tested as a matter of routine although not suffering overtly from lymphogranuloma, as well as the positive fixation by the serum of one worker in our laboratory and 55% of syphilitic sera which we have tested, the likelihood of undiagnosed or subclinical infection appears great and is being further investigated in collaboration with Dr. A. W. Grace.

If the reaction is specific, as we believe, it will prove to be a most

useful method for the detection of lymphogranuloma venereum infection particularly in individuals who have not been tested with Frei antigen, as well as for immunological studies in infections of humans and animals with this etiological agent.

Summary. In individuals with lymphogranuloma venereum, the serum has been found to fix complement regularly in the presence of antigens containing the virus in high concentration. Such fixation was observed only once with sera taken from supposedly uninfected individuals but was obtained frequently in syphilitic sera showing markedly positive Wassermann reactions.

11474

Persistence of St. Louis Encephalitis Virus in the Brains of Chicks.*

HAROLD E. PEARSON (Introduced by E. W. Schultz)

From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

St. Louis encephalitis virus has been cultivated *in vitro* on minced chick embryonic tissue¹ as well as in the yolk² and on the chorio-allantoic membrane^{1, 2-5} of chick embryos. On chorio-allantoic membranes, serial passage through 68³ and more than 100⁶ transfers has been possible. Brains of the corresponding embryos have been found to contain slightly more virus than the membranes, virus being also present in the livers and spleens.^{3, 5} Virus has been demonstrated in the brains of chicks allowed to hatch¹ though only slight or no microscopic changes were demonstrable.^{1, 3} It has been observed that embryos may survive until the time of hatching,³ or die within a few days after inoculation.^{1, 6}

Harrison and Moore¹ have reported that young chicks (4 to 6

^{*}Studies supported by Mary Hooper Somers Fund for Filtrable Virus Research.

¹ Harrison, R. W., and Moore, E., Am. J. Path., 1937, 13, 361.

² Stimpert, F., Proc. Soc. Exp. Biol. and Med., 1939, 41, 483.

³ Smith, M. G., Proc. Soc. Exp. Biol. and Med., 1939, 40, 191.

⁴ Smith, M. G., and Lennette, E. H., Proc. Soc. Exp. Biol. and Med., 1939, 41, 323.

⁵ Schultz, E. W., Williams, G. F., and Hetherington, A., Proc. Soc. Exp. Biol. AND Med., 1938, 38, 799.

⁶ Schultz, E. W., et al., unpublished work.

was taken as the highest dilution showing complete or nearly complete fixation with specific antigen and no fixation with the antigen control prepared from the corresponding normal tissues. The results with "Lygranum" (Y.S.) ran entirely parallel to those obtained with "Lygranum" (M.L.), although under the conditions of concentration employed in these experiments, the former antigen seemed more active. On the other hand, non-specific fixation in low serum dilutions with the antigen control from normal tissue was less frequently encountered in the case of mouse-lung. In performing the test the lowest initial serum dilution was 1:2 and since, in the system for fixation, this underwent a further threefold dilution, the lowest possible titre in these experiments was 1:6.

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If the reaction is specific, as we believe, it will prove to be a most

mouse brain passage virus. White leghorn, barred rock and Rhode Island red chicks were tested for susceptibility. The presence of virus in brains, livers or spleens of the chicks was determined by inoculating 3 mice each intracerebrally with 0.03 cc of a suspension (usually 10%) of material. Surviving test mice were observed for 21 days. All virus suspensions were also tested for bacterial sterility.

Webster's No. 3 strain of the virus was used. It regularly killed mice when inoculated intracerebrally in a dilution of 10⁻³.

Of forty 2-day-old leghorn chicks which were inoculated subdurally with 0.03 cc of a 10% suspension of mouse brain virus, none showed any sign of infection. The object of the investigation then was to determine whether or not the brains harbored active virus. The chicks were killed 2 at a time at intervals of several days over a period of 56 days. The brains of the 2 chicks were pooled, ground in a mortar and 3 mice were inoculated with each dilution. In some cases one brain only was ground, while the other was sectioned for microscopic examination. The results of the tests for virus are given in Table 1.

Histological examination of the brains of 11 chicks, not tested for virus, but killed on the 2nd to 20th day after inoculation showed only slight, and inconstant changes, consisting at most of small, perivascular, mononuclear infiltrations. The livers, spleens, and kidneys of these animals in all cases appeared normal. No changes were observed in the brains and other tissues from chicks killed more than 20 days after inoculation.

Groups of 4 to 6 chicks, 2 days old, were then inoculated by various routes with 10% mouse brain virus. None of these showed any sign of infection. They were killed at intervals and the tissues pooled and tested for virus. No gross changes were noted in any of the tissues. Microscopic sections of brains were stained by Lentz A method, other tissues with hematoxylin and eosin. The observations on this group are given in Table II.

Ten leghorn chicks, 21 days old, were inoculated subdurally, each with 0.03 cc of 10% mouse brain virus. These showed no sign of infection over a period of one month.

Serial passage of virus was tried (using leghorns for the first 2 passages and barred rocks subsequently) in chicks 2 to 6 days old. Three chicks were inoculated subdurally each with 0.03 cc of 10% mouse brain virus. These were killed 3 to 4 days later. Their brains were ground together to make a 10% suspension and 0.03 cc were inoculated subdurally into each of 3 new chicks and intra-

days old) were found somewhat susceptible. After inoculation with mouse brain virus, 3 out of 6 showed clinical (paralysis) or microscopic evidence of infection, or both, and virus was recovered from the brains of 3 out of 4 of the chicks tested for virus.

The present report deals with the susceptibility of young chicks to

TABLE I.
Results Obtained on Inoculating Suspensions of Chick Brains into Mice.

	Thocharing Suspension	s of Chiek Brains into Mice.
Days after inoculation chicks were killed	Dilution of chick brain employed	Death of mice in days
2	1:10 1:100	6 6 7 6 6 6
ភ	1:10 1:100 1:1000 1:10,000	6 8 — 8 10 —
4	1:10 1:100	$\begin{array}{cccc} 5 & 7 & * \\ 5 & 5 & 12 \end{array}$
4	1:10 1:100 1:1000	<u>e</u> — — — — — — — — — — — — — — — — — — —
G	1:10 1:100	4 7 11 7 8 -
G	1:10 1:100 1:1000	8 10 * 6 8 8
ន	1:10 1:100	6 = =
10	1:10 1:100	5 8 8 8 — -
10	1:10 1:100	<u> </u>
12	1:10 1:100 1:1000	$\begin{array}{cccc} 7 & 8 & 9 \\ 11 & 16 & 2 \\ \hline \end{array}$
12	1:10	
17	1:10	10 — —
20	1:10	8 10
21 31 42 56	1:10	= = =

Six survivors were discarded.

⁻ Survived.

^{*} Accidental death.

mouse brain passage virus. White leghorn, barred rock and Rhode Island red chicks were tested for susceptibility. The presence of virus in brains, livers or spleens of the chicks was determined by inoculating 3 mice each intracerebrally with 0.03 cc of a suspension (usually 10%) of material. Surviving test mice were observed for 21 days. All virus suspensions were also tested for bacterial sterility.

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Groups of 4 to 6 chicks, 2 days old, were then inoculated by various routes with 10% mouse brain virus. None of these showed any sign of infection. They were killed at intervals and the tissues pooled and tested for virus. No gross changes were noted in any of the tissues. Microscopic sections of brains were stained by Lentz A method, other tissues with hematoxylin and eosin. The observations on this group are given in Table II.

Ten leghorn chicks, 21 days old, were inoculated subdurally, each with 0.03 cc of 10% mouse brain virus. These showed no sign of infection over a period of one month.

Serial passage of virus was tried (using leghorns for the first 2 passages and barred rocks subsequently) in chicks 2 to 6 days old. Three chicks were inoculated subdurally each with 0.03 cc of 10% mouse brain virus. These were killed 3 to 4 days later. Their brains were ground together to make a 10% suspension and 0.03 cc were inoculated subdurally into each of 3 new chicks and intra-

Results Obtained When Chicks Were Ingeniated with a 10% Suspension of Mouse Brain Vinus TABLE II.

Subcutancously 0.3 cc 0.03 cc 0.03 cc 0.003 cc	No. of chicks inoculated	5 Leghorns	4 Barred Rocks	5 Barred Rucks	6 R. I. Reils	6 R. 1. Reds
30 28 28 21 (a) (a) (b) ains 2 brains 3 brains 3 brains 3 brains 4 brains 4 brains 4 brains 5 brains 6 b (b) (c) (c) (d)	Route and amount inoculated	0.3 co snbeufancously	0.03 cc subdurally	0.3 ec intraperitoneally	0,03 (c subdurally	0.4 ec intraperitonvally
3 hains 2 hains 3 brains 3 brains 3 brains 3 brains (1/2 of each) 4 phens) 4 phens) 4 phens) (b) (c) (d)	Days after inoculation chicks killed	30	S S1	801	21	21
3 (livers 3 (livers 4 livers 4) (1/2 of each) splicers) splicers) (b) (c) (d)	Results on inoculating	3 hains	(a) 2 bains	3 brains	3 brains	3 հումու
(b) (c) (d)	trsucs into mice were negative. Kind of tissue (pooled)	3 (livers spirens)	3 (livers spleems)	3 (livers	(½ of each)	3 (livers spiecens)
	Microscopic examination of tissues of chicks which were not tested for virus	(p)	(p)	(0)	(q)	(2)

 (b) No changes abserved in brains, livers in spheris
 (c) Our brain had a small area of monounchar cell infiltration in the choroid plexies; a second had a similar area but perivascular in distribution. (d) Of 3 brains scettoned (in this ense hulf of each brain was tested for virus), 2 showed several small foed of monumuelear cell infiltration, mostly in the recebellum at the level of Purkinge cells. (e) Brains not examined. Invers and spiceus showed no changes.

				FAB	LE	III.				
No. of the serial passage		1			2		***************************************	3	į	5
Death of individual mice in days	5	5	5	G	6	7	s	\$ 10	19 — —	7 7 12
No. of the serial passage		ij			7			\$	9 10 days	9 25 days
Death of individual	4	9	14	9	10	•	7	7 7	7 8	

⁻ Sarvival

cerebrally into 3 mice. None of the chicks showed any sign of infection. Six instead of 3 chicks were used in the 9th passage: 3 of these were killed 10 days and three 25 days after inoculation. The results of the mouse inoculation with material from each serial chick passage are given in Table III.

Microscopic sections of the 3 brains of chicks from the 9th passage sacrificed 10 days after inoculation showed several foci of mononuclear cell infiltration near the meninges, some diffuse mononuclear cell infiltration and perivascular infiltration in various areas and particularly in the cerebellum.

Serial passage in chicks 2 to 6 days old was also attempted, in which the initial inoculation consisted of 0.5 cc of a 10% suspension of mouse-brain-virus administered intraperitoneally to each of 3 chicks. Transfers were then made by grinding all the brains and spleens together to make a 10% suspension and inoculating each of 3 new chicks with 0.5 cc intraperitoneally and 3 mice each with 0.03 cc intracerebrally. None of the chicks showed any sign of infection. Virus was recovered from the first passage, but not from the subsequent 8 passages.

Summary. Two-day-old chicks, after inoculation with St. Louis encephalitis virus, subdurally or by other routes, failed to show any clinical evidence of infection. Nevertheless, the brains of chicks which had been inoculated subdurally proved infectious for mice in a dilution of 10° for at least 6 days and sometimes in a dilution of 10° up to 20 days. Histological sections of such brains showed only slight changes, consisting at most of small areas of perivascular infiltrations.

The virus was carried through nine serial passages at 3- to 4-day intervals in the brains of 2- to 6-day-old chicks. Chick brains of the ninth serial passage showed areas of focal, diffuse and perivascular infiltration.

^{*} Accidental death

11475

Effect of Breathing Pure Oxygen on Respiratory Volume in Humans.*

NATHAN W. SHOCK AND MAYO H. SOLEY

From the Institute of Child Welfare, the Divisions of Physiology, Medicine, and Pharmacology of the Medical School, University of California

Although previous investigators have concluded that the respiratory volume is not altered by the inspiration of high concentrations of oxygen^{1, 2} we have found an increase in the average respiratory volume in normal males when pure oxygen was breathed.

A group of 33 white male students, ages 18-33, served as subjects (mean age 23.5±.8). Each subject was given a preliminary trial on the day before the experiments were conducted and was then tested with oxygen at the corresponding hour of 2 subsequent days. In each experiment the subject rested for 20 minutes in the supine position and no tests were run sooner than one hour following a meal. Expired air was collected through a Siebe-Gorman halfmask and mercury valves (opened by a pressure of 1.5 mm of water), into a pair of spirometers of the Tissot type, each with a capacity of 9.19 liters (at 0°, 760 mm). The apparatus, which is described in detail elsewhere³ was arranged so that the time was electrically recorded when 9.19 liters of air were expired. Expired air was measured continuously over a period of 15 minutes before the administration of the pure oxygen and for 30 minutes during the inspiration of oxygen.

Oxygen was obtained in 1150-gallon pressure tanks from which a pair of Tissot spirometers, each with a capacity of 60 liters, was filled. In this way the oxygen was allowed to come to the same temperature and pressure as room air before being breathed. The valve system was arranged so that the change in inspired air from 21% to 100% oxygen could be made without the knowledge of the subject. Each experiment consisted of: (1) a 20-minute rest period in the supine position; (2) a 15-minute period for the measurement of respiratory volume with the subject breathing outdoor air; (3)

^{*} Aided by a grant from the Research Board and the Christine Breon Fund for Medical Research of the University of California. Grateful acknowledgment is made to the Work Projects Administration (O.P. 65-1-08-62, Unit A-8) for clerical assistance in the preparation of this material.

¹ Benedict, F. G., and Higgins, H. L., Am. J. Physiol., 1911, 28, 1.

² Davies, H. W., Brow, G. R., and Binger, C. A. L., J. Exp. Med., 1925, 41, 37.

³ Shoek, N. W., and Ogden. E. Child Development. In press.

a 20-minute period for the measurement of respiratory volume with the subject breathing 100% oxygen. In 36 experiments a fourth period of 15 minutes of air collection was continued with the subject again breathing outdoor air from the larger spirometers.

Respiratory volumes were computed in liters per minute at 0° C and 760 mm Hg. The resting level was determined for each experiment from the average of at least 8 observations. The change in respiration resulting from breathing pure oxygen was expressed as

TABLE I. Effect of Breathing 100% O_2 on Resting Respiratory Volume

		Resti Test I.	ng Resp	iratory Vol	unte. Test II.		I, and II
	Breathing	······································		Breathing	Breathing		Avg.
	21% 0,	100% O.	%	21% 02	100% 0.	%	%
Subject	1./sq m/	1./sq m/	Incie-	1./sq m/	1./sq m/	Incre-	Incre
No.	min	min	ment	min	min	ment	ment
7	2.95	3.14	6	2.87	2.75	4	1
8 9	3.80	4.11	8	4.02	4.13	3	6
9	4.16	4.03	- 3	5.41	5.88	9	3
10	2.97	3.04	2	3.32	3.73	13	8
11	3.75	4.35	16	4.38	5.14	17	17
12	3.65	4.11	12	3.31	3.64	10	11
13	4.12	4.32	5	3.85	3.98	3	4
14	4.16	4.39	6	3.64	4.10	13	10
15	3.08	3.45	12	2.95	3.28	11	12
16	3.26	4.02	23	3.33	3.95	18	21
17	3.56	4.85	36	3.45	4.17	21	29
18	2.55	2.62	3	2.41	2.51	4	4
19	3.10	4.26	37	3.48	4.46	28	33
20	4.67	3.73	-20	4.10	4.27	4	- 8
21	3.18	4.08	28	3.12	3.63	16	22
22	3.38	4.04	20	3.39	4.29	26	23
23	2.52	2.89	32	2.29	2.62	14	23
24	3.67	4.57	24	3.88	4.52	17	21
25	2.52	2.89	15	2.46	3.06	24	20
26	2.98	4.93	GG	3.63	3.41	- 6	30
27	4.01	4.23	5	3.78	4.30	14	10
28	3.84	3.97	3	4.21	4.42	5	4
29	2.48	3.02	22	2.59	2.83	9	16
30	2.66	3.29	24	2.83	3.48	23	24
31	3.52	3.70	5	3.32	3.36	1	3
32	2.97	3.05	3	3.07	2.94	- 4	- 1
33	3.59	4.15	15	3.61	3.90	8	$\frac{12}{5}$
34	3 06	3.26	7	3.35	3.41	ž	5
35	2.55	2.59	1	2.47	2.46	0	1
36 27	2.70	3.60	33	3.39	3.64	7	20
37	3.25	3.31	2	2.92	3.56	22	12
38	3.30	3.60	9	2.60	3.99	54	32
39	3.46	3.77	9	3.35	4.02	20	15
Mean	3.32	3.75	14.1	3.36	3.75	12.2	13.4
					S.D	. Mn. C.R.	1.8 7.5

11475

Effect of Breathing Pure Oxygen on Respiratory Volume in Humans.*

NATHAN W. SHOCK AND MAYO H. SOLEY

From the Institute of Child Welfare, the Divisions of Physiology, Medicine, and Pharmacology of the Medical School, University of California

Although previous investigators have concluded that the respiratory volume is not altered by the inspiration of high concentrations of oxygen^{1, 2} we have found an increase in the average respiratory volume in normal males when pure oxygen was breathed.

A group of 33 white male students, ages 18-33, served as subjects (mean age 23.5±.8). Each subject was given a preliminary trial on the day before the experiments were conducted and was then tested with oxygen at the corresponding hour of 2 subsequent days. In each experiment the subject rested for 20 minutes in the supine position and no tests were run sooner than one hour following a meal. Expired air was collected through a Siebe-Gorman halfmask and mercury valves (opened by a pressure of 1.5 mm of water), into a pair of spirometers of the Tissot type, each with a capacity of 9.19 liters (at 0°, 760 mm). The apparatus, which is described in detail elsewhere was arranged so that the time was electrically recorded when 9.19 liters of air were expired. Expired air was measured continuously over a period of 15 minutes before the administration of the pure oxygen and for 30 minutes during the inspiration of oxygen.

Oxygen was obtained in 1150-gallon pressure tanks from which a pair of Tissot spirometers, each with a capacity of 60 liters, was filled. In this way the oxygen was allowed to come to the same temperature and pressure as room air before being breathed. The valve system was arranged so that the change in inspired air from 21% to 100% oxygen could be made without the knowledge of the subject. Each experiment consisted of: (1) a 20-minute rest period in the supine position; (2) a 15-minute period for the measurement of respiratory volume with the subject breathing outdoor air; (3)

^{*} Aided by a graut from the Research Board and the Christine Breon Fund for Medical Research of the University of California. Grateful acknowledgment is made to the Work Projects Administration (O.P. 65-1-08-62, Unit A-8) for clerical assistance in the preparation of this material.

¹ Benedict, F. G., and Higgins, H. L., Am. J. Physiol., 1911, 28, 1.

² Davies, H. W., Brow, G. R., and Binger, C. A. L., J. Exp. Med., 1925, 41, 37.

³ Shock, N. W., and Ogden. E. Child Development. In press.

Although Dennis and Bolton' suggested certain advantages of the thermo-coagulation method for the induction of lesions in the rat's brain, they made no attempt to describe in detail the procedures used to induce circumscribed lesions of varying magnitudes and depths in the brains of small animals frequently used in laboratories. present technic, in brief, has involved the application of a heated platinum wire to a selected area of the exposed skull bone. This wire is left upon the skull for an interval, the magnitude of which depends upon the nature and purpose of the experiment. Microscopic study of the cerebral tissue, following sectioning and staining, indicates clearly that differential destruction of specific cortical layers may be effected by varying the duration of the application of the heated cautery wire to the external surface of the skull. Careful observation of other layers of the cerebrum indicates that the lesions so induced are clearcut and that adjacent cells are normal in appearance.

The data available have been accumulated from the study of the cerebral areas of 35 rats. These animals, all male albinos, were 3 months of age at the time that the cortical operations were performed. The results indicate that the application of the heated cautery tip to the skull for a period of 10 seconds effects the destruction of the outer or first cellular layer of the cortex within the auditory area. If the wire is applied for 20 seconds all cortical layers within this region underlying the tip are destroyed. With an interval of 15 seconds the first five layers are destroyed. Twenty-five and 30-second intervals induce well delimited lesions which extend into the hippocampal regions. A detailed analysis of other areas and the intervals essential for the induction of cortical lesions of differing depths is in progress.

The cautery unit utilized for the induction of these brain lesions by thermo-coagulation has been devised for use in dentistry.* The heat obtained from this instrument is generated by alternating current. The unit is equipped with a dial which can be set for the regulation of the degree of heat generated by the electric current passing through the cautery tip acting as a resistance. A more accurate quantification of the power dissipated in the cautery tip has been made by means of the voltmeter-animeter method. The calories of heat per second generated by the cautery tip have been determined for each dial reading.

¹ Dennis, W., and Bolton, C., Science, 1935, 81, 297.

² Pennington, L. A., J. Comp. Neurol., 1937, 66, 415.

^{*} Cautery unit devised and patented by Burton Manufacturing Company, Chicago,

a percentage deviation from the resting value obtained in the period prior to the breathing of oxygen. Since similar results were obtained when computations were based on the period of breathing air after the administration of oxygen, it is clear that the results cannot be attributed to increased restlessness of the subjects during a prolonged experiment.

Results are shown in Table I. It may be seen that in all but 5 experiments in the total of 66 an increase in respiratory volume occurred with the inspiration of pure oxygen. The average increment was 13.4% for 66 experiments with 33 subjects. Statistical tests indicate that this average increment would occur by chance only once in 1×10^{-4} trials.

The cause of this increase in respiration is speculative but the following possibilities are suggested: (1) because of the increased oxygen tension in the blood, less oxyhemoglobin is reduced in the tissues, thus releasing less base for CO₂ transport from the tissues. In this way the CO₂ tension of the respiratory center itself may be increased with a resulting increase in respiratory volume; (2) the increase in oxygen tension of the blood reduces cerebral blood flow which may result in a local increase in CO₂ in the respiratory center; (3) an increase in oxygen tension in the respiratory center may increase the sensitivity of the center to the normal stimulus so that respiratory volume is increased although no rise in [H+] or pCO₂ occurs.⁶

Summary. Breathing pure oxygen causes a significant rise in the average resting respiratory volume in normal males.

11476 P

Thermo-coagulation in Destruction of Tissue in Cerebral Cortex of Small Animals.

L. A. PENNINGTON (Introduced by J. F. Fulton) From the Psychological Laboratory, University of Illinois.

In order to overcome certain mechanical difficulties inherent in the trephining method when applied particularly to the removal of tissue in the none too accessible auditory areas of the rat's cerebral hemispheres, the technic about to be described was devised.

⁴ Gesell, R., Am. J. Physiol., 1923, 66, 5.

⁵ Lennox, W. G., and Gibbs, E. L., J. Clin. Invest., 1932, 11, 1155.

⁶ Eastman, W. J., International Clinics, Series 46, 1936, 11, 275.

11477

Respiration of Kidney Cortex in High Potassium-Low Sodium Ringer's Solution.*

ALVIN E. LEWIS AND JOHN FIELD, 2D. From the Department of Physiology, Stanford University.

Carr and Beck¹ have shown that in the brief life span of an albino rat after bilateral adrenalectomy there is a gradual decrease of about 25% in the basal metabolic rate, when the animal is maintained on a "normal" diet. The experiments reported here are part of a series designed to determine the cause and effect relationship of this decreased metabolic rate in the syndrome of adrenal insufficiency. In this instance an attempt was made to see whether the typical serum electrolyte picture in advanced adrenal insufficiency would reduce the respiration of kidney slices from normal rats. The kidney was chosen for study because of its importance in producing the adrenal insufficiency syndrome² and because it has been shown by Crismon and Field³ that there is a decrease in kidney respiration in the adrenalectomized rat of about 38%.

Kidney slices were prepared with the Terry razor microtome which proved very satisfactory for this purpose. The oxygen consumption of kidney cortex was measured by the Warburg method The suspension medium used in control experiments was the mammalian Ringer's of Dickens and Greville, hereinafter termed D G-Ringer's. For the experimental series the medium was a high potassium-low sodium modification of mammalian Ringer's solution osmotically balanced with glucose, hereinafter called A.I.-Ringer's The concentrations of electrolytes in this medium were those reported for blood of adrenalectomized animals by Grollman' and Hegnauer and Robinson The compositions of these solutions are summarized in Table I.

^{*} Supported in part by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

¹ Carr, C J, and Beck, F. F., Am. J. Physiol., 1937, 119, 589.

Loeb, R. F, Glandular Physiology and Therapy, Cincago, The American Medical Association, 1935, Chapter 20.

Crismon, J. M., and Field, J., .im. J. Physiol., 1940, in press.

⁴ Terry, B. T , Am. J. Clin. Path., 1937, 7, 69.

⁵ Field, J., Belding, H. S., and Martin, A. W., J. Cell. Comp. Physiol., 1939, 14, 143

⁶ Dickens, F , and Greville, G. D., Biochem. J., 1935, 29, 1468.

⁷ Grollmin, A., The Adrenals, Biltimore, Williams and Wilkins Co., 1936, 184 188.

⁸ Hegn wer, A. H , and Robinson, E. J., J. Biol. Chem , 1936, 116, 769.

Additional comments concerning the behavioral data obtained from this study are relevant at this point. These data obtained from carefully controlled observations of the animals in an auditory discrimination apparatus³ were in general agreement with those obtained from the observations of other animals operated upon by the extirpation method. Although these 2 sets of operative and behavioral data were not strictly comparable because of differential extents and depths of the lesions, it is clear that the method of thermo-coagulation results in postoperative disturbances in animal behavior similar in degree to those observed in animals of the other group.

That the values of this method are numerous seems clear. First, recovery of the animal is rapid. If the investigator wishes, the behavior of the animals may, in most instances, be studied from one to three days after operation. Rapid recovery makes possible a more detailed and complete collection of experimental data pertaining to the problem of the restitution of function following artificial injuries to the nervous system. Second, the procedure requires far less time for the actual performance of the operation, and, hence tends to reduce operative shock. This method does necessitate, however, the aid of an assistant who serves as a timekeeper. Third, the approach makes readily accessible, in the rat brain at least, certain cerebral areas, especially areas j, k, and p delimited earlier by Fortuyn.4 Fourth, the method results in fewer cases of infection and thus reduces the mortality rate. Fifth, the thermo-coagulation technic is more readily controlled than is the trephine method in studies on small animals. It makes possible the induction of very small or of very large cerebral lesions with fewer technical difficulties than is ordinarily possible with the extirpation method currently in use.

³ Hunter, W. A., and Pennington, L. A., Science, 1939, 89, 87.

Fortuyn, A. B. D., Arch. Neurol. and Psychiat., London, 1914, 6, 221.

11477

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ALVIN E. LEWIS AND JOHN FIELD, 2D. From the Department of Physiology, Stanford University.

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² Loeb, R. F., Glandular Physiology and Therapy, Chicago, The American Medical Association, 1935, Chapter 20.

³ Crismon, J. M., and Field, J., Am. J. Physiol., 1940, in press.

⁴ Terry, B. T., Am. J. Clin. Path., 1937, 7, 69.

⁵ Field, J., Belding, H. S., and Martin, A. W., J. Cell. Comp. Physiol., 1939, 14, 143.

⁶ Dickens, F., and Greville, G. D., Riochem. J., 1935, 29, 1468.

⁷ Grollman, A., The Adrenals, Baltimore, Williams and Wilkins Co., 1956, 184-188.

⁸ Hegnauer, A. H., and Robinson, E. J., J. Biol. Chem., 1936, 116, 769.

TABLE I.

	D.GRinger* g per liter	A.IRinger* g per liter
NaCl	7.00	5.4
KCl	0.18	0.36
MgCl ₂ ,6H ₂ O	0.1627	0.1956
CaCl ₂ ,2H ₂ O	0.25	0.25
Glucose	2.00	9.80

^{*}Both solutions were buffered at pH 7.4 with sodium phosphate in final concentration of M/150.

Thirty-two determinations were made in D.G.-Ringer's solution and 39 in the A.I.-Ringer's solution. Since the tissues were obtained from 9 white rats (Slonaker-Wistar strain), the arithmetic means of the results obtained on each animal were analyzed statistically as paired data. The mean oxygen consumption, N.P.T., per mg (dry weight) in one hour was 16.954 cu mm in D.G.-Ringer's solution and 16.098 cu mm in A.I.-Ringer's solution. The mean difference was 0.855; the standard deviation was 1.29; the standard error was 0.489, and the value of "t" (Fisher, 1936) was 1.7485. This indicates that this small difference in oxygen consumption could occur by chance more than 5 times in a hundred. Thus, the difference observed is not statistically significant. Although the mean difference observed may actually exist, as might possibly be shown by a more lenient statistical method, this observed fall in oxygen consumption, even at its maximum, is not of the order of magnitude of that observed for tissues of adrenalectomized animals.

It can readily be seen that this experiment represents a very limited reproduction of the situation occurring in adrenal insufficiency. Although the electrolyte content of the A.I.-Ringer's solution approximates the blood picture occurring in fairly extreme cases of adrenal insufficiency, there are certain aspects of the total picture which have necessarily been omitted. Thus, for example, the time factor has been completely neglected. Whereas the electrolyte change occurring in adrenal insufficiency is slow and chronic, this experiment, as a first approach to the evaluation of the electrolytic factors, necessarily represents an acute situation.

To the extent that the experimental situation produced here in vitro is comparable to the electrolytic imbalance occurring in adrenal insufficiency it would appear that the changed electrolyte content does not have a direct rôle of significant magnitude in the depression of the oxygen consumption of the kidney cortex.

11478

Influence of Rate of Urine Formation on Potassium Excretion.*

V. E. HALL AND L. L. LANGLEY

From the Department of Physiology, Stanford University.

From an accurate description of the relations existing between the rate of renal excretion of any substance and the rate of urine formation, it is possible to make certain deductions concerning the manner in which the kidney excretes that substance. In spite of the active current interest in potassium metabolism, this relation as it exists in man has not been satisfactorily studied. We have attempted to fill this gap.

Repeated simultaneous determinations of plasma and urinary potassium concentrations were made upon 3 normal adult male subjects maintained on diets of approximately constant potassium content. The water content was varied within wide limits. With extreme water deprivation, urine flow rates as low as 0.5 cc per min. were obtained; while with ingestion of excess water, the flow reached 6 or more cc per min. Urine was collected for one 2-hour period, in the middle of which blood was drawn for analysis. The blood was oxalated and centrifuged immediately at 3000 rpm for 20 minutes. Potassium was determined on ashed urine and plasma by the method of Kramer and Tisdall.^{1, 2} Special precautions were taken to prevent loss of precipitate in washing. The subjects carried on regular laboratory work during the experimental periods.

The excretion rates have been expressed as clearances (C), calculated by the conventional formula:

$$C = \frac{U V}{P}$$

in which U is the concentration of potassium in the urine (mg per cc). I' the volume of urine (cc per min.), and P the plasma potassium concentration (mg per cc). The plasma potassium concentrations (means with standard errors of means) were: Subject L, 16.4 ± 0.2 ; Subject C, 16.5 ± 0.6 ; and Subject H, 17.9 ± 0.4 mg per 100 cc.

From Fig. 1, in which the clearances are plotted against the rates of urine flow, it may be seen that, over a wide range of flow rates,

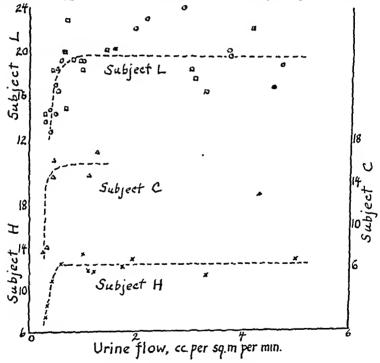
^{*} Supported in part by a grant from the Fluid Research Fund of the Stanford University School of Medicine.

¹ Kramer, B., and Tisdall, F. F., J. Biol. Chem., 1921, 46, 339.

² Tisdall, F. F., and Kramer, B., J. Biol. Chem., 1921, 48, 1.

the clearance remains constant. Below about 0.6 cc per sq m per min ("the augmentation limit"), the clearance falls sharply in all 3 subjects. Since, according to Chesley, the glomerular filtration rate begins to fall off as the urine flow reaches approximately this value, it is probable that the decrease in potassium clearance at urine flow rates below the observed augmentation limit is due to a reduction in glomerular filtration.

The observations of Griffon' apparently showed that the rate of potassium excretion in man was proportional to the rate of urine flow. However, he confined his observations to the range of 0.35 to 1 cc per min., and was thus working largely below the augmentation limit. On the other hand, the data of Cutler, Power and Kendall' suggested that in normal human subjects potassium excre-



Effect of urine flow rate on potassium clearance in normal male subjects. Clearances (ordinates, with separate scales for each subject) are stated as ce plasma cleared per min.

³ Chesley, L. C., J. Clin. Invest., 1938, 17, 591.

⁴ Griffon, H., Comp. rend. Soc. de Biol., 1936, 121, 47.

⁵ Cutler, H. H., Power, M. H., and Wilder, R. M., J. Am. Med. Assn., 1938, 111, 117.

tion was independent of urine flow. All the flow rates of these investigators now appear to have been above the augmentation limit. Our observations confirm and reconcile the findings of both these

groups of workers.

The clearance ("maximal") of our 3 subjects averaged about 16 cc per sq m per min. Since the glomerular filtration rate of normal subjects is about 69 cc per sq m per min, potassium must be reabsorbed by the kidney tubules. Since both the rate of glomerular filtration and the rate of potassium excretion remain constant over a wide range of urine flow rates, the rate of tubular reabsorption must also remain constant. Accordingly, the concentration of potassium in the tubular urine may vary widely without causing changes in the rate of tubular reabsorption. Such reabsorption cannot therefore be entirely a passive process resulting from the gradient established by the reabsorption of water.

The factors controlling such reabsorption are now under investigation in this laboratory.

11479 P

Hemolytic Action of Fluorides on Certain Nucleated Erythrocytes.

T. N. HAMDI AND J. K. W. FERGUSON From the Marine Biological Laboratory, Woods Hole, Mass.

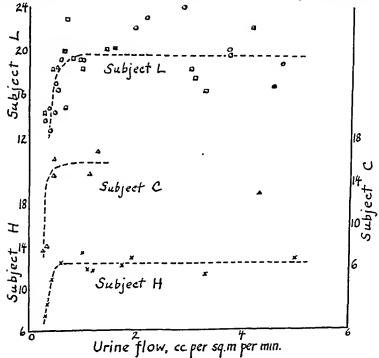
In a study of CO₂ dissociation curves of dogfish blood (Ferguson, Horvath and Pappenheimer¹ it was observed that sodium fluoride added to prevent glycolysis caused a slowly progressive swelling, usually ending after 3 to 10 hours in hemolysis. Hemolysis of fish blood by oxalate has been observed by Black and Irving.² The effects of several fluorides, oxalates and other salts have been tested on the blood of various species with nucleated erythrocytes. One tenth molar and molar solutions of the salts were added to 9 times their volume of the blood to be tested, which had previously been defibrinated. The final concentration of the salts was, in one series, one-hundredth molar and, in the other, one-tenth molar. In the first series the final solution bathing the cells would be slightly

¹ Ferguson, J. K. W., Horvath, S. M., and Pappenheimer, J. R., Biol. Bull., 1938, 75, 381,

² Black, E. C., and Irving, Laurence, J. Cell. Comp. Physiol., 1938, 12, 255.

the clearance remains constant. Below about 0.6 cc per sq m per min ("the augmentation limit"), the clearance falls sharply in all 3 subjects. Since, according to Chesley, the glomerular filtration rate begins to fall off as the urine flow reaches approximately this value, it is probable that the decrease in potassium clearance at urine flow rates below the observed augmentation limit is due to a reduction in glomerular filtration.

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³ Chesley, L. C., J. Clin. Invest., 1938, 17, 591.

⁴ Griffon, H., Comp. rend. Soc. de Biol., 1936, 121, 47.

⁵ Cntler, H. H., Power, M. H., and Wilder, R. M., J. Am. Med. Assn., 1938, 111, 117.

11480

Effect of Arsenicals on Liver Lipids of Rabbits.

P. L. MacLachlan. (Introduced by E. J. Van Liere.)

From the Department of Biochemistry, School of Medicine, West Virginia
University, Morgantown.

Attempts to determine the effect of liver injury on the amount and distribution of the liver lipids have yielded widely divergent results. Theis' found that the relation of phospholipid to neutral fat is quite constant for normal liver tissue and may be expressed as an equilibrium, 55 to 60% phospholipid: 45 to 40% neutral fat. However, if the liver is damaged or diseased this relation is altered. abnormal organs seldom show any change from normal in the amount of total lipid, but the proportion of phospholipid is greatly diminished apparently because of a failure to convert neutral fat to phospholipid. Results obtained by MacLachlan2 for white rats are at variance with those reported by Theis in two respects: (1) the proportion of total lipid present as phospholipid in normal liver tissue is considerably higher, and (2) no displacement of the normal phospholipid: neutral fat balance takes place as a result of liver injury. MacLachlan and Hodge³ found in cocaine-fed mice which showed extensive liver injury that the neutral fat and cholesterol contents increase greatly but the phospholipid content remains strikingly constant. This clearly shows that a change in the phospholipid to neutral fat ratio of the liver lipids from normal may result from a change in the neutral fat content only.

Since arsenicals are capable of producing extensive necrosis of the liver with fatty degeneration, it was considered worthwhile to determine the effect of arsphenamine and neoarsphenamine poisoning on the amount and distribution of the liver lipids.

Fourteen young adult rabbits of both sexes were maintained on a diet of Purina rabbit chow for 2 weeks prior to the experiment. To each of 4 rabbits, 50 mg per kg of arsphenamine were administered intravenously every third day until 5 doses were given: to each of another 2 animals. 5 doses of 75 mg per kg of neoarsphenamine were administered similarly. Thus the rabbits received a total of 250 mg per kg of arsphenamine or 375 mg per kg of neoarsphenamine within 2 weeks. The remaining 8 animals served as controls.

¹ Theis, E. R., J. Biol. Chem., 1928, 76, 107; 1928, 77, 75; 1929, 82, 327.

² MacLachlan, P. L., Proc. Soc. Exp. Biol. and Med., 1936, 34, 31.

³ MacLachlan, P. L., and Hodge, H. C., J. Biol. Chem., 1939, 127, 721.

hypotonic and in the second slightly hypertonic. In the later experiments using molar solutions the more alkaline ones (oxalates, citrates and arsenates) were adjusted by the addition of HCl to a pH between 7.0 and 7.4, as indicated by phenol red. This procedure produced in certain cases significant alterations in the results. The following salts were tested on all the species used—sodium chloride, sodium thiocyanate, sodium fluoride, sodium oxalate. In some experiments the following salts were used, too—sodium citrate, sodium arsenate (mostly dibasic), aluminium fluoride, zinc fluoride. The bloods tested included dogfish (Mustelus cauis), tautog (Tautoga ouitis), sea robin (Prionotus caroliuus), squeteague (Cyonosciou regale) and a turtle and a snake (species unknown).

Results. Progressive swelling was not observed after the addition of sodium chloride or sodium thiocyanate. All the fluorides tested produced progressive swelling and (when the observations were sufficiently prolonged) hemolysis of the erythrocytes of all the fish listed above. The nucleated erythroctyes of the turtle and snake were not, however, susceptible to fluoride. The results with oxalate, citrate and arsenate were less regular. Sometimes swelling and hemolysis were produced and it occurred more often with the stronger concentrations. In a few experiments it appeared that even the stronger solutions failed to cause swelling when they were neutralized. This phenomenon suggested a possible explanation of the results.

Fluorides, oxalates, arsenates and citrates might be expected to remove magnesium and calcium ions from solution. The latter 3 anions would remove magnesium less effectively in neutral or acid solution, although they would still be effective in removing calcium ion. The removal of magnesium ion from the susceptible bloods might alter the permeability of erythrocytes in some manner which would result in the swelling and hemolysis e.g. by increasing the permeability to cations. However, these results are presented in this incomplete form not so much to indicate an explanation as to draw attention to a phenomenon which has complicated investigations of the transport of respiratory gases by fish blood. Circumstances make it unlikely that we shall be able to pursue this investigation further in the near future.

Summary. The nucleated erythrocytes of certain fishes show progressive swelling and eventual hemolysis on the addition of fluorides. Oxalates and arsenates produce a similar effect but with less regularity. The nucleated erythrocytes of a turtle and a snake did not react in this way.

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Attempts to determine the effect of liver injury on the amount and distribution of the liver lipids have yielded widely divergent results. Theis' found that the relation of phospholipid to neutral fat is quite constant for normal liver tissue and may be expressed as an equilibrium, 55 to 60% phospholipid: 45 to 40% neutral fat. However, if the liver is damaged or diseased this relation is altered. abnormal organs seldom show any change from normal in the amount of total lipid, but the proportion of phospholipid is greatly diminished apparently because of a failure to convert neutral fat to phospholipid. Results obtained by MacLachlan2 for white rats are at variance with those reported by Theis in two respects: (1) the proportion of total lipid present as phospholipid in normal liver tissue is considerably higher, and (2) no displacement of the normal phospholipid: neutral fat balance takes place as a result of liver injury. MacLachlan and Hodge³ found in cocaine-fed mice which showed extensive liver injury that the neutral fat and cholesterol contents increase greatly but the phospholipid content remains strikingly This clearly shows that a change in the phospholipid to neutral fat ratio of the liver lipids from normal may result from a change in the neutral fat content only.

Since arsenicals are capable of producing extensive necrosis of the liver with fatty degeneration, it was considered worthwhile to determine the effect of arsphenamine and neoarsphenamine poisoning on the amount and distribution of the liver lipids.

Fourteen young adult rabbits of both sexes were maintained on a diet of Purina rabbit chow for 2 weeks prior to the experiment. To each of 4 rabbits, 50 mg per kg of arsphenamine were administered intravenously every third day until 5 doses were given; to each of another 2 animals, 5 doses of 75 mg per kg of neoarsphenamine were administered similarly. Thus the rabbits received a total of 250 mg per kg of arsphenamine or 375 mg per kg of neoarsphenamine within 2 weeks. The remaining 8 animals served as controls.

¹ Theis, E. R., J. Biol. Chem., 1928, 76, 107; 1928, 77, 75; 1929, 82, 327.

² MacLachlan, P. L., Proc. Soc. Exp. Biol. and Med., 1936, 34, 31.

³ MacLachlan, P. L., and Hodge, H. C., J. Biol. Chem., 1939, 127, 721.

A small portion of each liver was used for moisture determination. Lipid analyses were made on another portion by standard procedures, Bloor and Boyd.

Histological examination of the livers showed moderate to severe necrosis with fatty degeneration as a result of the arsenical treatment. However, the results of the chemical analyses of the livers (Table I) show that there are no significant changes from normal following the administration of either arsphenamine or neoarsphenamine. The neutral fat content of the treated animals, while showing more individual variation, is no greater in amount than that of the untreated animals. The constancy of the phospholipid content, moreover, does not support the idea that in liver injury neutral fat increases at the expense of phospholipid. The normal values obtained for the total lipid content and the ratio of phospholipid to neutral fat of the livers of rabbits following arsenical poisoning are in agreement with the observations of MacLachlan² for rats following liver injury, but stand in marked contrast to the findings of MacLachlan and Hodge^a for cocaine-fed mice. Apparently a change in the phospholipid: neutral fat balance of the liver

TABLE I.

Liver Lipids of Rabbits Following Administration of Arsphenamine and Neoarsphenamine. (Calculated on the basis of moist weight.)

Rabbit .	Moisture, %	Total Lipid, %	Phospho- lipid,	Neutral Fat,	Chole- sterol,	Phospholipid : Neutral Fat† %
1.C	71,9	4.42	3.15	.928	.337	71:21
$\tilde{2}\cdot\tilde{\mathbf{C}}$	71.1	4.68	3.43	.873	.381	73:19
3-Č	71.7	4.68	3.68	.666	.331	79:14
4.C	72.0	4.44	3.44	.669	.335	78:15
5-C	73.3	3.95	3.07	.462	.415	78:12
6-C	70.7	4.23	3,45	.487	.297	82:12
7.C	71.5	4.01	3.22	.516	.278	80:13
8-C	70.4	4.15	3,23	.569	.350	78:14
8-0						
Avg	71.6	4.32	3,33	.646	.341	77:15
9-A	73.7	4.12	3.16	.361	.600	77: 9
10-A	74.3	4.11	3.44	.237	.433	84:6
11-A	72.0	4.43	3.62	.372	.437	82: 8
12-A	70.5	3.74	2.47	.995	.277	66:26
13-N	72.0	4.74	3.29	.960	.390	70:20
14·N	72.5	4.04	3.03	.645	.365	75:16
Avg	72.5	4.20	3.17	.595	.417	76:14

^{*}C-Control; A-Arsphenamine; N-Neoarsphenamine.

Expressed as per cent of total lipid.

⁴ Bloor, W. R., J. Biol. Chem., 1928, 77, 53.

⁵ Boyd, E. M., J. Biol. Chem., 1931, 91, 1.

following liver injury occurs only when there is a change (from normal) in the total lipid content of the organ.

Summary. Liver injury in rabbits resulting from the administration of arsenicals in the form of arsphenamine and neoarsphenamine cause no significant changes from normal in the amount or distribution of the liver lipids.

The author wishes to express his appreciation to Dr. G. A. Emerson of the Department of Pharmacology for furnishing the experimental material and to Dr. J. E. Andes of the Department of Pathology for the histological examination of the tissues.

11481

Effect of Chlorination of City Water on Virus of Poliomyelitis.*

J. EMERSON KEMPF AND MALCOLM H. SOULE From University of Michigan, Ann Arbor.

Water was considered in early reports concerning the transmission of poliomyelitis. This method of spread seemed unlikely when later experimental evidence favored an air-borne infection entering the host through the olfactory tract. However, Kling, observing European epidemics, reconsidered the question and additional evidence was accumulated incriminating water as a factor in the spread of the virus.

Poliomyelitis virus was found in human feces as early as 1912² and these observations have been amply confirmed. Unfortunately the technic of Sawyer³ requiring a second monkey passage as an important criterion to verify the presence of the virus was ignored until 1938. In that year, Trask, Vignec, and Paul.⁴ and Kramer, Hoskwith, and Grossman⁵ improved the technic of virus isola-

^{*} This work was aided by a grant from the Clara Ward Seabury Chine for the Study of Infantile Paralysis.

We are indebted to Mr. Harry McEntee, Supervising Chemist, Ann Arbor Water Softening Plant, for valuable assistance in this work.

¹ Kling, C., Bull. Office internat. d'hyg. pub., 1928, 20, 1779.

² Khug, C., Petterson, A., and Wernstedt, W., Communication Inst. méd. Ltat, Stockholm, 1912, 3, 5.

³ Sawyer, W. A., Am. J. Trop. Dis. and Prev. Med., 1915, 3, 164.

⁴ Trask, J. D., Viguec, A. J., and Paul, J. R., Proc. Soc. Exp. Biol. and Med., 1938, 38, 147.

⁵ Kramer, S. D., Hoskwith, B., and Grossman, L. H., J. Exp. Med., 1939, 69, 49.

tion and included serial passage in monkeys. Using the new procedure Paul, Trask, and Gard^a detected the virus in sewage in the Charleston and Detroit epidemics of 1939. In addition, Kramer, Gilliam and Molner⁷ isolated the virus from stools of healthy contacts in a Detroit institutional outbreak. Others⁸⁻¹² during the past year have succeeded in isolating the virus and carrying it through a second animal passage.

The presence of the virus in human intestinal discharges led Levaditi, Kling and Lépine¹³ to investigate the effect of chlorination. A concentration of 4 parts per million (ppm) destroyed the virus in a cloudy tap water emulsion of infected monkey cord in 24 hours; 0.40 ppm was equally effective with clarified preparations. The pH and temperature of the emulsions were not recorded. They concluded that chlorination by the usual methods was virucidal. This work is lacking in two essentials, namely, the minimum effective chlorine concentration, and the shortest effective contact period. The chlorine concentrations and contact periods they used were considerably in excess of those employed in this country. Because of lack of data on the virus-inactivating effects of chlorination as usually practiced, the problem was reinvestigated.

Fresh water was obtained for each experiment from the Ann Arbor Water Softening Plant where the water is treated by the ammonia-chlorine process in which chlorine is present as chloramines. The chlorine content of the water was determined by the ortho-tolidine test. Reducing substances in the water did not interfere with its accuracy. The MV virus was selected for these experiments. It had a minimal infective dose of approximately 0.001 g of spinal cord.

The suspensions to be tested were prepared by making a 10% emulsion of infected spinal cord in saline with subsequent centrifugation at 4,500 rpm (radius 10 cm). The supernatant contained a minimum amount of organic matter. This was desirable since

⁶ Paul, J. R., Trask, J. D., and Gard, S., J. Bact., 1940, 39, 63.

⁷ Kramer, S. D., Gilliam, A. G., and Molner, J. G., Public Health Rep., 1939, 54, 1914.

⁸ Lépine, P., and Sédallian, P., Comp. rend., 1939, 208, 129.

⁹ Toomey, J. A., Arch. Ped., 1939, 56, 693.

¹⁰ Howe, H. A., and Bodian, D., Proc. Soc. Exp. Biol. and Med., 1939, 41, 538.

¹¹ Stimpert, F. D., personal communication.

¹² Kempf, J. E., and Soule, M. H., unpublished.

¹³ Levaditi, C., Kling, C., and Lépine, P., Bull. Acad. de méd., Paris, 1931, 105, 190.

Mallman¹⁴ and Fox¹⁵ demonstrated a protective effect on bacteria of organic matter in the presence of chlorine.

In experiment 1, 2.0 cc of the supernatant were added to 100.0 cc of chlorinated water and the same amount to a distilled water control. The initial chlorine content at the time of adding the virus was 0.58 ppm; this dropped to 0.10 ppm in an hour at which time 2.0 cc of each solution were injected intracranially into monkeys. Both animals developed quadriplegia, the control in 6, the other in 8 days.

Experiments 2 and 3 (Tables I and II): One part of the virus suspension was diluted with 165 parts of chlorinated water; a distilled water control was also prepared. Five minutes later the chlorine content, temperature and pH were determined. At stated intervals, monkeys were inoculated as in Experiment 1. The temperature readings were made at the time of each monky injection.

Attention should be directed to the persistence of a chlorine

TABLE I. Effect of Chlorination on MV Virus in 1:1650 Dilution.

	Chlorine	Chlorine concentration, ppm					
Monkey No.	Original Cone,	5 min afte exposure to virus	r At time of inoculation	Contact period, hr	Neurological signs	Time of onset, days	
3	(Control)	***************************************			Quadriplegia*	12	
4	0.55	0.55	0.50	11.2	,,,	35	
5	0.55	0.55	0.35	4	Negative		
6	0.53	0.55	0.20	10	,,		
7	0.55	0.55	0.05	24	* * * * * * * * * * * * * * * * * * * *		

^{*}Histopathological picture was compatible with that of acute poliomyelitis. †Temperature 21.24°C; pH 8.5.

TABLE 11. Effect of Chlorination on MV Virus in 1:1650 Dilution.

	Chlorin	e concentr:	ation, ppm			Time of onset, days
Monkey No.	Original Conc.		r At time of inoculation	Contact period, hr	Neurological signs	
8	(Control)				Quadriplegia*	10
9	0.80	0.55	0.40	1	,,,	14
					XI nerve paraly	sis*
10	0.80	0.55	0.40	214	Leg paralysis	10
**					Arm paresis	
11	0.80	0.55	0.25	3	Negative	

^{*}Histopathology was compatible with that of acute poliomyelitis. †Temperature 21-23° C; pH 8.3.

¹⁴ Mallman, W. L., Mich. Eng. Exp. Sta., Bull. No. 59, 1934.

¹⁵ Fox, L. A., Military Surgeon, 1936, 78, 329.

content of 0.50 ppm for 1½ hours in Experiment 2, indicating a negligible chlorine demand by the organic matter. In Experiment 3, apparently there was more organic material present because the residual chlorine dropped from 0.80 ppm to 0.50 in 5 minutes and to 0.40 ppm in 1 hour. The contact period required for inactivation of the virus was approximately the same in both instances. As an additional control 0.45 ppm was adequate to kill B. coli in a concentration of 24,000 organisms per cc in ½ hour.

In municipal practice, a residual chlorine content of 0.10 to 0.20 ppm for ½ to 2 hours is considered adequate for the production of a safe water. The results in this paper indicate that a higher concentration and a longer contact period are necessary to inactivate the virus of poliomyelitis. The possibility that drinking water, adequately chlorinated according to accepted standards, may be a factor in the epidemiology of poliomyelitis must be recognized as a result of these findings. As a corollary, attention is directed to the shortcoming of this method for the protection of swimming pool water since carriers may discharge the virus from the intestinal tract or the naso-pharynx and the chlorine content of swimming pools is apt to drop significantly during the peak bathing loads. The need for more sensitive methods for detecting the poliomyelitis virus in water should be emphasized. Even persistently negative results would not necessarily assure the absence of the virus from water, because organisms such as B. typhosus are seldom found by direct bacteriological methods.

Whether the aluminum hydroxide sedimentation process previous to chlorination would produce virus-free water cannot be answered in this paper. Experiments are being continued to determine whether the chlorine concentrations usually used in swimming pools are sufficient to inactivate the virus.

Summary. Chlorine in a concentration of 0.5 ppm, which is an amount in excess of that usually employed in municipal practice, did not inactivate the virus of poliomyelitis in 1½ hours.

11482

Non-Induced Cardiopathic Disease in a Rabbit—Electrocardiographic and Pathologic Study.

JAN NYBOER. (Introduced by Maurice Bruger.)

From the Department of Medicine, New York Post-Graduate Medical School and Hospital, Columbia University, New York City.

The recognition of heart disease in experimental animals would be a great asset in the selection of healthy animals. Miller¹ has shown that spontaneous interstitial myocarditis existed in rabbits. Reference to the electrocardiographic diagnosis of non-induced cardiac disease in laboratory animals was not found in the literature. However, many electrocardiographic studies on induced heart disease have been made.²-⁴ Seifried⁵ referred to pathologic studies in rabbits with heart disease.

In a preliminary control electrocardiographic study on 16 three-month-old rabbits, there was a definite variation in the voltage, rhythm and form of the electrical complexes. The electrocardiogram of rabbit No. 7 in this series diverged definitely from the average so that a diagnosis of acute myocardial disease was suggested. This tracing also indicated the possibility that the disease might be localized in the myocardium.

This rabbit was received in a shipment one week previously and no known experiments had been performed on it. General observations showed the animal to be drowsy, inactive, and anorexic. The rectal temperature was 103° F. on the day the electrocardiogram was taken. Inanition continued for 2 days. On the third, the rabbit was found dead in its cage. On autopsy, a gross inspection of the body, lungs and abdominal viscera showed no demonstrable pathology. The epicardium, however, was adherent to the right anterolateral chest wall. No pericardial effusion was present. By comparison with normal hearts it measured about the same size. The heart was placed in formalin, sectioned and stained with hemotoxylin and eosin for microscopic study.

Figure I. shows electrocardiograms of a control rabbit and of

¹ Miller, C. P., J. Exp. Mcd., 1924, 40, 524.

² Agduhr, E., and Stenstrom, N., The Appearance of the Electrocardiogram in Heart Lesions Produced by Cod Liver Oil Treatment, Almquist and Wiksells, Uppsala, 1930.

³ Johnston, F. D., Hill, I. G. W., and Wilson, F. N., Am. Heart J., 1935. 10, 903.

⁴ Wood, F. C., and Wolferth, C. C., Arch. Int. Mcd., 1933, 51, 771.

⁵ Seifried, O., Kranketten des Kannichens, Julius Springer, Berlin, 1937.

content of 0.50 ppm for 1½ hours in Experiment 2, indicating a negligible chlorine demand by the organic matter. In Experiment 3, apparently there was more organic material present because the residual chlorine dropped from 0.80 ppm to 0.50 in 5 minutes and to 0.40 ppm in 1 hour. The contact period required for inactivation of the virus was approximately the same in both instances. As an additional control 0.45 ppm was adequate to kill B. coli in a concentration of 24,000 organisms per cc in ½ hour.

In municipal practice, a residual chlorine content of 0.10 to 0.20 ppm for 1/2 to 2 hours is considered adequate for the production of a safe water. The results in this paper indicate that a higher concentration and a longer contact period are necessary to inactivate the virus of poliomyelitis. The possibility that drinking water, adequately chlorinated according to accepted standards, may be a factor in the epidemiology of poliomyelitis must be recognized as a result of these findings. As a corollary, attention is directed to the shortcoming of this method for the protection of swimming pool water since carriers may discharge the virus from the intestinal tract or the naso-pharynx and the chlorine content of swimming pools is apt to drop significantly during the peak bathing loads. The need for more sensitive methods for detecting the poliomyelitis virus in water should be emphasized. Even persistently negative results would not necessarily assure the absence of the virus from water, because organisms such as B. typhosus are seldom found by direct bacteriological methods.

Whether the aluminum hydroxide sedimentation process previous to chlorination would produce virus-free water cannot be answered in this paper. Experiments are being continued to determine whether the chlorine concentrations usually used in swimming pools are sufficient to inactivate the virus.

Summary. Chlorine in a concentration of 0.5 ppm, which is an amount in excess of that usually employed in municipal practice, did not inactivate the virus of poliomyelitis in $1\frac{1}{2}$ hours.



A section through rabbit No. 7 myocardium, approximately midway between the apex and the nuricular junction. Low power magnification.

The chief objective differences between these graphs were defined by describing the deviations in the abnormal electrocardiogram of rabbit No. 7. The presence of the Q_1 of 2.8 mm, the depressed RS- T_1 of 1.7 mm, the inverted \widetilde{T}_1 of 1.7 mm, the elevated RS- T_3 of 1.4 mm were probable deviations from the normals, but the T_2 and T_3 in the standard leads were upright and not definitely

rabbit No. 7. The control animal exhibited only slight variations in the contours of its electrocardiograms during 4 months of observation. Significant variations in the standard leads (I, II, III) and of the exploring right and left chest leads paired with the indifferent left leg electrode were observed in rabbit No. 7 as compared with the control observations.

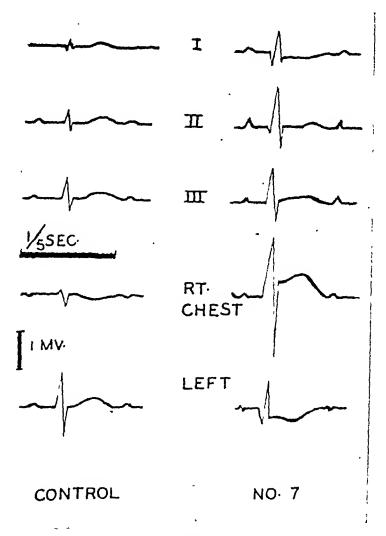


Fig. 1.

Diagram of a normal (control) and the abnormal rabbit electrocardiograms (rabbit No. 7) taken in all leads at normal sensitivity and adapted to the new terminology for the exploratory leads.



A section through rabbit No. 7 myocardium, approximately midway between the apex and the auricular junction. Low power magnification.

The chief objective differences between these graphs were defined by describing the deviations in the abnormal electrocardiogram of tabbit No. 7. The presence of the Q_t of 2.8 mm, the depressed RS- T_t of 1.7 mm, the inverted T_t of 1.7 mm, the elevated RS- T_t of 1.4 mm were probable deviations from the normals, but the T_t and T_t in the standard leads were upright and not definitely

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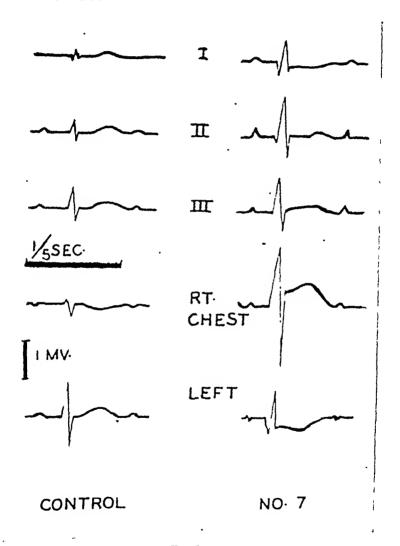


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Microscopic sections through different levels of the myocardium of a normal rabbit showed the muscle bundles sharply outlined and the nuclei well stained. No evidence of degeneration or inflammation was present. Sections through the myocardium of rabbit No. 7 showed (Plates 1 and 2) areas of necrosis everywhere, chiefly affecting the muscle and largely involving the right ventricle and interventricular septum. In the left ventricle the areas of necrosis were chiefly beneath the endocardium. The papillary muscles were prominently affected. Slight periarterial infiltration was present and areas of necrosis were seen in both the auricular walls. The anterolateral ventricular epicardium showed a hyaline fat necrosis. The pathological diagnosis was degeneration and necrosis of the myocardium.

Summary. The incidence of non-induced cardiopathic disease among laboratory animals may greatly alter the prognosis, course and reaction to given control or experimental conditions. Disease of the myocardium as confirmed by pathologic studies may greatly alter the electrocardiogram in the rabbit. The changes found suggesting a localized lesion of the ventricle by the electrocardiogram were not supported by pathologic studies. Since bacteriologic studies were not done, no conclusive evidence as to the etiology of the myocarditis was suggested.

11483

Variability of Action on Heart Rate Compared with Metabolic Effect of Various Thyroid Preparations.

ARTHUR E. MEYER AND H. DANOW

From the Research Laboratory of The Maltine Company, Brooklyn, New York.

In our previous publication¹ we have shown that certain thyroid preparations fed to thyroidectomized rats exert a stimulating action on the heart rate which varied from one product to the other in its relation to the corresponding metabolic increase obtained. While in 2 U.S.P. thyroid preparations the cardiac effect prevailed, thyroxine and thyroid globulin proved to be of low action on the heart if given at a dose to produce an equal metabolic response. It was shown furthermore, that alkaline hydrolysis of thyroid globulin

¹ Meyer, A. E., and Yost, M., Endocrinology, 1939, 24, 806.

abnormal. The right chest exploring lead showed no evidence of a Q wave, but a markedly elevated RS-T segment, associated with an upright T wave. The left chest exploring lead showed a deep Q wave and a markedly depressed RS-T segment associated with an inverted T wave. These RS-T deviations are definitely abnormal. The voltages of the chief QRS deflections in all the leads appeared greater than those observed in normal rabbits. In general, the rate of 300 per minute was faster than the average of normal rabbits studied.

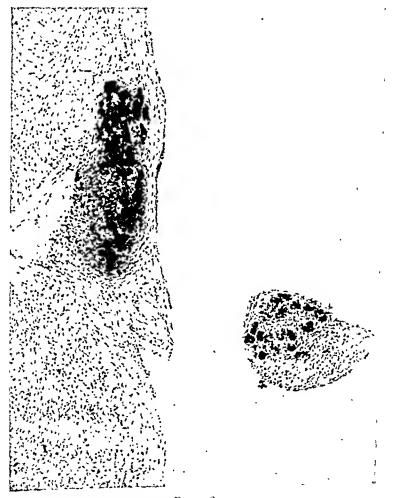


PLATE 2.

Magnification of Plate 1 section in the marked inset showing scattered areas of normal, necrobiotic, and necrotic tissue beneath the endocardium and of the papillary muscle.

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¹ Meyer, A. E., and Yost, M., Endocrinology, 1939, 24, 806.

caused the formation of 2 split products, representing essentially the thyroxine and diiodotyrosine fractions, which differed widely in the degree of their metabolic potency, but which both showed a quite conspicuous action on the heart. It was concluded that hydrolysis produced a heart stimulator from either the thyroid hormone itself or from some unspecific substance contained in the material subjected to hydrolyzing agents. The question whether or not the heart stimulation obtainable with U.S.P. thyroid was caused by a substance contained originally in the gland, or to some split product formed by post-morten changes could not be decided upon.

In continuation of this work we compared a number of other thyroid preparations, either dried gland powders of different commercial provenience or extracts prepared from thyroid, with respect to metabolic effect and action on heart rate, using the same technic as described before, which consisted in feeding the medication (calculated in gamma per 10 g body weight) for 3 days and determining the metabolic and heart effect on the fifth day.

Samples of dried whole thyroid (U.S.P.) used in our previous work gave the standard metabolic response of 30% increase at a dosage of 310 to 320 γ , corresponding to 0.62 to 0.64 γ of iodine. In the following experiments the dosage producing 30% metabolic

		TABLE 1.		
Product tested	Actual fodine content of undiluted product,	Quantity of material after dilution to 0.2% I content, producing 27-32% metabolic stimulation	Quantity of thyroxine contained in quantity given in	Avg heart stimulation obtained at about 30% ine. of metabolism, Before After Incr.
d,l-thyroxine as Na salt	65.0	244	.73	$190 \cdot 220 = 30$
Thyroid Globulin		0.00	-20	200 010 20
No. 123	.565	260	.23	$180 \cdot 210 = 30$
134	.78	293	,25	$190 \cdot 226 = 36$
136	.72	310	.22	$188 \cdot 136 = 48$
141	.44	275	.27	190 - 227 = 37
143	.76	289	.26	190 - 212 = 22
U.S.P. A.	.2	320	.30	$185 \cdot 340 = 155$
W-1	.465	309	.25	190 - 365 = 175
U.S.P. W-2	.2	240	.19	192 - 310 = 118
W-3	.63	236	.20	195 - 284 = 89
U.S.P. C.	.23	320	.26	$192 - 275 \pm 83$
U.S.P. L.	.2	320		190 - 273 = 83
Thyroid Ext. P.	.3	300		190 - 275 = 85
Commercial Thyroid Pro	tein .94	446	.3	195 - 260 = 65

² Meyer, A. E., and Wertz, A., Endocrinology, 1939, 24, 683.

response was determined in every instance and the effect on the heart rate obtained simultaneously was noted.

Since not all these preparations were U.S.P. and some had a higher iodine content, the products were diluted with milk-sugar to contain 0.2% iodine in order to obtain comparable figures.

The table giving the averages, obtained on 6-12 rats in each case, shows that the metabolic effect in a large percentage of preparations is proportionate to the iodine content, the standard dose being about 300 γ , but that deviations even in U.S.P. thyroid do occur, as shown in sample W-2 and W-3. The effect on the heart was not in proportion with the metabolic efficiency, confirming our previous findings that both effects are to some extent independent.

The commercial thyroid protein, claimed to be "detoxified," gave a relatively low heart stimulation but a 50% higher dosage was required for the standard metabolic effect. The thyroxine content of the products was determined by the Leland-Foster method.³ From the data presented in the table the ratio between thyroxine and metabolic effect seems to be slightly more variable than that between iodine and that action, the quantity in the dosage varying from 0.19 to 0.3 γ .

Incidentally, iodized protein and peptone supplied by Dr. W. T. Salter, Thorndike Memorial Laboratory, Boston, gave proportionate metabolic responses in agreement with clinical tests but induced practically no heart acceleration. 5. 5

The observation mentioned above that hydrolysis of the thyroid globulin produces split products of strong action on the heart was met with the criticism that this effect might be due to some unspecific product of decomposition obtainable by hydrolysis from any animal tissue and perhaps present in the commercial product of high effect on the heart as a consequence of autolytic changes in the structural elements of the gland tissue occurring before the drying process was completed.

To answer the question beef muscle was minced and subjected to hydrolysis. The water-insoluble part was extracted with alcohol and both aqueous and alcoholic extract combined and evaporated. The extract did not show any sign of metabolic effect when tested on rats nor did it affect the heart rate. It was admixed to a standardized thyroid globulin that at 85 γ dosage per 10 g given for 3 days produced a metabolic increment of 30% and heart rate increase of about 25 beats per minute. The figures obtained by the

³ Leland, J. P., and Foster, G. L., J. Biol. Chem., 1932, 95, 165.

⁴ Lerman, J., and Salter, W. T., PROC. Soc. Exp. Biol. and Med., 1938, 38, 94.

⁵ Salter, W. T., and Lerman, J., Trans. Assn. Am. Phys., 1938, 53, 202.

caused the formation of 2 split products, representing essentially the thyroxine and diiodotyrosine fractions, which differed widely in the degree of their metabolic potency, but which both showed a quite conspicuous action on the heart. It was concluded that hydrolysis produced a heart stimulator from either the thyroid hormone itself or from some unspecific substance contained in the material subjected to hydrolyzing agents. The question whether or not the heart stimulation obtainable with U.S.P. thyroid was caused by a substance contained originally in the gland, or to some split product formed by post-mortem changes could not be decided upon.

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TABLE	I.

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Product tested	%	γ	<u> </u>	Before After Incr.
d.l-thyroxine as Na salt Thyroid Globulin	65.0	244	.73	190 - 220 = 30
No. 123	.565	260	.23	$180 \cdot 210 = 30$
134	.78	293	.25	$190 \cdot 226 = 36$
136	.72	310	.22	$188 \cdot 136 = 48$
141	.44	275	.27	$190 \cdot 227 = 37$
143	.76	289	.26	$190 \cdot 212 = 22$
U.S.P. A.	.2	320	.30	$185 \cdot 340 = 155$
W·1	.465	309	,25	190 - 365 = 175
U.S.P. W-2	,2	240	.19	$192 \cdot 310 = 118$
W-3	,63	236	.20	195 - 284 = 89
	.23	320	.26	$192 \cdot 275 = 83$
U.S.P. C.	.2	320		$190 \cdot 273 = 83$
U.S.P. L.	.3	300		$190 \cdot 275 = 85$
Thyroid Ext. P. Commercial Thyroid Pro		446	.3	$195 \cdot 260 = 65$

² Meyer, A. E., and Wertz, A., Endocrinology, 1939, 24, 683.

Results. Initial body weights of the control $(45.7\pm2.3~\mathrm{g})$ and test groups $(45.2\pm1.6~\mathrm{g})$ showed no significant difference $(0.5\pm2.8~\mathrm{g})$. The observed difference of $19.7\pm4.7~\mathrm{g}$ between the final body weights of the test $(193.8\pm3.8~\mathrm{g})$ and control $(174.1\pm3.0~\mathrm{g})$ animals in favor of the treated group, however, was probably significant. Likewise, the difference in final body length of $12.0\pm3.6~\mathrm{mm}$ in favor of the treated group $(203\pm2.8~\mathrm{mm})$ as compared to the controls $(191\pm2.7~\mathrm{mm})$ was also probably significant.

The significant increase in body weight and length of animals treated with small doses of testosterone propionate stands in contrast to the growth-inhibiting influence of large doses of this same hormone.² A statement concerning the effect on length is included although initial body lengths were not taken. This was deemed permissible since the test and control animals had originally shown no significant difference in body weight. Body lengths, which are normally so highly correlated with body weights³ may therefore be assumed to have been approximately similar before treatment was begun. The gain in body weight of approximately 11% may not appear very large but when one recalls that growth curves of animals treated with substances lacking growth-stimulating properties remain essentially parallel⁴ and the difficulties encountered in trying to stimulate growth during the early age period of the albino rat,⁵ any gain must be considered seriously.

Conclusions. Testosterone propionate administered intraperitoneally to male albino rats in doses of 0.05 mg daily (except Sunday) from 26 to 80 days of age led to a probably significant increase in body weight and length. This growth-stimulating effect of small doses of testosterone propionate stands in contrast to the growth-depressing effect of large doses of the same hormone.

² Rubinstein, H. S., Kurland, A. A., and Goodwin, M., Endocrinology, 1939, 25, 724.

³ Donaldson, H. H., The Rat, Memoirs of the Wistar Institute of Anatomy and Biology, Philadelphia, 1924.

⁴ Rubinstein, H. S., J. Comp. Neur., 1936, 64, 3.

⁵ Rubinstein, H. S., Bull. Sch. of Mcd., University of Maryland, 1933, 17, 163.

use of the mixture were absolutely identical. The conclusion, therefore, is justified that the heart stimulator is not a split product obtainable by hydrolysis from this type of animal tissue.

Conclusions. The iodine content in thyroid preparations seems to be an approximate guide for the estimation of metabolic effect; however relatively large deviations do occur in some products. The effect on the heart is not related to the metabolic action. The thyroxine content has still less demonstrable proportionality to either physiologic effect. While hydrolysis of thyroid globulin increases its heart action hydrolysate from muscle tissue is inert in that respect.

11484 P

Growth-Stimulating Effect of Testosterone Propionate.*

H. S. RUBINSTEIN AND M. L. SOLOMON.

From the Research Laboratory, Surgical Division, Sinai Hospital, Baltimore, Md.

For this purpose 24 male albino rats of Wistar Institute strain were used. Of these 12 animals were treated (test group) and 12 served as untreated controls. All animals were kept under similar conditions, Purina Dog Chow used as food, and water were constantly present. In addition, green vegetables were given twice weekly. The treated animals received daily (except Sunday) intraperitoneal injections of 0.05 mg testosterone propionate (Perandren) for 53 days beginning at 26 days of age. Control animals remained uninjected.

Weights were taken at 26 days of age and weekly thereafter. Twenty-four hours after the last injection, i.e. at 80 days of age, all animals were anesthetized with ether, their carotid vessels were cut and exitus was allowed to result from bleeding. Body lengths measured from the tip of the snout to the anus were then determined.

All data were treated statistically and observed differences between test and control groups were considered as being probably significant only if the "significance ratio" was 3 or more.

1 Pearl, R., Medical Biometry and Statistics, second edition, Saunders, Phila-

delphia, 1930.

^{*} The authors gratefully acknowledge the aid of the Ciba Pharmaceutical Products Company, Inc., for partially defraying the expenses of this study and for furnishing the testosterone propionate (Perandren) used.

injected under pressure into the quarter and allowed to remain until the next milking. Within one hour after the injection, the treated quarter became distended and the rectal temperature began to increase, reaching 41°·C at the 5th or 6th hour. The temperature returned to nearly normal in about 3 hours thereafter, and the acute swelling had about subsided at the next milking.

Repeated treatments of the 2 animals mentioned above failed to

Repeated treatments of the 2 animals mentioned above failed to eliminate permanently the streptococci from the infected quarters. This may be explained in part by the inadequacy of the method of administration of the bactericidal substance and also by the fact that these 2 animals were well advanced in their lactation periods and that the infected quarters were severely indurated.

Three cows less advanced in the lactation period were selected for the following tests. Nine infected quarters were treated, one of which had been inoculated artificially and allowed to carry an infection for 17 days before treatment. Repeated treatments failed to eliminate the streptococci from 2 quarters. Five treatments were required to sterilize one of the quarters which was moderately indurated. These repeated treatments stimulated the production of fibrosis and resulted in a decrease in milk-secretion. The streptococci disappeared from the other 6 quarters (in 5 cases after a single treatment) without an appreciable decrease in milk production. The fact that streptococci had been eliminated was established by daily bacteriological examination of the milk over periods ranging from 15 to 81 days.

Before the effectiveness of gramicidin in the control of bovine mastitis can be determined, a larger number of animals must be treated and observed over a longer period of time. The influence of fibrosis, the state of the lactation, the competency of the closing mechanism of the teat, and other factors will have to be considered. While the streptococci were not eliminated from all of the treated quarters, they were markedly decreased after each treatment, and the findings thus confirm the results obtained in mice, namely, that gramicidin, when injected directly into an infected focus, exhibits a definite bactericidal effect against streptococci.

11485 P

Action of Gramicidin on Streptococci of Bovine Mastitis.

R. B. LITTLE, R. J. DUBOS, AND R. D. HOTCHKISS

From the Department of Animal and Plant Pathology and the Department of the Hospital, The Rockefeller Institute for Medical Research, Princeton, N. J., and New York, N. Y.

Granicidin—an alcohol-soluble, water-insoluble substance isolated from cultures of a sporulating bacillus—has been shown to exert a marked bactericidal effect against gram-positive microorganisms, both in vitro and in vivo. It has been found for instance that 0.002 mg of this substance injected intraabdominally into white mice, exerts a therapeutic action against experimental peritonitis caused by pneumococci and streptococci; gramicidin, however, has proved almost completely ineffective when administered by the intravenous, intramuscular, or subcutaneous route.¹⁻³

It is known that in the chronic form of bovine mastitis caused by Streptococcus agalactiæ (Lancefield group B), the infection is confined to the infected quarter of the udder and rarely results in a demonstrable systemic disturbance. It appeared of interest, therefore, to determine whether gramicidin, when injected into the infected quarter, would destroy the streptococci causing the mastitis.

A number of cases of chronic mastitis were selected for study and it was established by daily bacteriological examination of the milk that the numbers of streptococci remained high (over 100,000 per cc of milk) during a period of several weeks prior to treatment.

The toxic reactions which result from the injection of gramicidin into the bovine udder and a convenient method of administration of the substance, were determined on 2 cows suffering from chronic mastitis. These animals received repeated treatments with increasing amounts of granicidin diluted in Ringer's solution, which proved very irritating, and later in distilled water, which was more satisfactory. The following technic was finally adopted for the treatment of each individual quarter. Gramicidin in amounts of 60 to 240 mg was diluted in 1000 cc of double distilled sterile water at 40° C. Following the morning milking, the residual milk in the cistern and in the teat was flushed out with 100 to 200 cc of a dilute solution of gramicidin; 800 to 900 cc of the preparation were then

¹ Dubos, R. J., J. Exp Med , 1939, 70, 11

² Dubos, R. J., and Cattaneo, C., J. Exp. Med., 1939, 70, 249

³ Hotchkiss, R D., and Dubos, R J , J Biol Chem , 1940, 182, 791

injected under pressure into the quarter and allowed to remain until the next milking. Within one hour after the injection, the treated quarter became distended and the rectal temperature began to increase, reaching 41°·C at the 5th or 6th hour. The temperature returned to nearly normal in about 3 hours thereafter, and the acute swelling had about subsided at the next milking.

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11486

A Comparison of Interstitial Cell-Stimulating, Ovarian-Stimulating, and Inhibiting Actions of Pituitary Glands of Different Species.

ELEANOR WEST AND H. L. FEVOLD.

From the Biological Laboratories, Harvard University.

There is very little data concerning the luteinizing hormone content of various kinds of pituitaries which are used in experimental work. It has not been accurately established in what amounts the gonad-stimulating hormones exist in the pituitaries of different species of animals. Recently a method of assay has been described which permits the accurate determination of the LH present in pituitary tissue in the presence of the other gonadotropic factor FSH.¹ In most assay methods the FSH acts synergistically with LH, and thus confuses the results.

Likewise, there are no data available regarding the capacity of pituitary glands from different species to inhibit the action of FSH in producing follicular development in the ovaries of immature rats. It has been variously reported (a) that this factor is separate and distinct from the follicle-stimulating and the luteinizing hormone^{2, 3} and (b) that it is the luteinizing hormone which produces this effect under the proper conditions.^{4, 5}

This paper reports the quantitative assay of the LH content and also the inhibiting action of the pituitary glands of sheep, hog, and beef. The potencies of these pituitary tissues in stimulating ovarian development in the immature female rat are also recorded to give a comparative idea of the FSH potency.

Methods of Assay. The increase in the weight of the seminal vesicles of immature male rats has been used as a measure of the luteinizing hormone, since it has been shown that the LH stimulates the production of male hormone in the male rat. It was also shown that FSH augments the action of LH in the production of male hormone, so that in its presence the results were not a true measure

¹ Fevold, H. L., J. Biol. Chem., 1939, 128, 83.

² Evans, H. M., Korpi, K., Pencharz, R. I., and Simpson, M. E., Univ. Calif., Pub. Anat., 1936, 1, 237.

³ Bunde, C. A., and Hellbaum, A. A., Am. J. Physiol., 1939, 125, 290.

⁴ Jensen, H., Simpson, M. E., Tolksdorf, S., and Evans, H. M., Endocrinology, 1939, 25, 57.

⁵ Fevold, H. L., and Fiske, V. M., Endocrinology, 1939, 24, 823.

⁶ Greep, R. O., Fevold, H. L., and Hisaw, F. L., Anat. Rec., 1936, 65, 261.

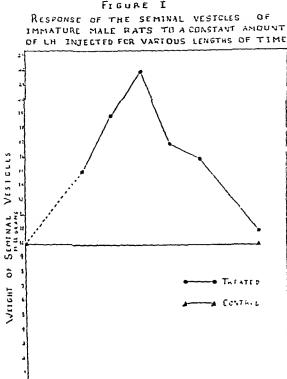


FIGURE I

of the LH. However, if the pituitary material is injected intraperitoneally, FSH is no longer effective, while LH is as active as when injected subcutaneously. It is thus possible to negate the augmenting action of FSH, and the LH activity can be accurately determined.

LENGTH OF TREATMENT

The pituitary extract was injected intraperitoneally twice daily, morning and evening, in 0.25 cc doses into immature male rats (21 days old) for 4 days, since it was found that injections of a constant amount of LH given for this length of time resulted in the maximum response of the seminal vesicles. (Fig. I.)

A unit of LH is taken as the smallest amount of pituitary tissue necessary to produce a 100% increase in the weight of the seminal vesicles of the injected animals over those of the uninjected controls.

The inhibiting potency of the pituitary glands was determined in the following manner. Enough FSH was given subcutaneously over a period of 4 days to immature female rats (21 days old) to produce an increase of 300% in the weights of the ovaries. The pituitary preparations to be tested were injected simultaneously intraperitoneally and an inhibiting unit was taken as that amount of pituitary material which would reduce the response to 150%.

The ovarian-stimulating potency was determined by injecting the preparations twice daily for 4 days into immature female rats (21 days old) and weighing the ovaries the morning of the fifth day. At the time of autopsy the ovaries were also observed to determine if they were primarily follicular or luteinized.

Preparation of Materials to be Tested. Acetone-desiccated pituitary powders or fresh pituitary tissue were extracted with an alkaline solvent at pH 8.0, and the extractives precipitated with acetone. The precipitate was thoroughly extracted with distilled water to remove the gonadotropic hormones, leaving as a residue the material rendered insoluble by the acetone precipitation, apparently because of denaturization. The aqueous extracts were then dried and stored as powders. This water-soluble material was always precipitated with tannic acid before injections, and injected as fine aqueous emulsions. In this manner the absorption rate should be as nearly equal from preparation to preparation, irrespective of the impurities, thereby avoiding one source of error.

Results. Table I presents the results of the assay of pituitaries of different species for the luteinizing hormone. It is at once apparent that sheep pituitary glands are the best source of LH and that beef pituitary glands contain the least. With the hog pituitary materials the results vary considerably, but in all cases the LH content is lower than in those of sheep. In 2 of the hog preparations very small amounts of LH were present, as indicated not only by the inability of these 2 preparations to stimulate male hormone secretion but also by the fact that the ovarian development, produced by these

TABLE I.

Interstitial Cell-Stimulating, Ovarian-Stimulating, and Inhibiting-Actions of
Pituitary Tissue of Sheep, Hog and Beef.

		Ovarian	Inhibiting	Ratios	
Preparation	LH content, Ru/Kg	development, Ru/Kg	action, Ru/Kg	LH/ov.	Lh/inhib
Sheep pit. powder	143,000	16,000		9.0	
Fresh sheep pit.	20,000	20,000		1.0	0.07
7,7	30,000	10,000	120,000	3.0	0.25
Hog pit. powder	2,000	12,500		0.16	
Fresh hog pit.	1,666	20,000		0.08	0.05
110311 1177 177	5,000	15,000	20,000	0.33	0.25
" beef "	1,666	625		2.6	
,, ,, ,,	2,000	666	10,000	1.6	0.2

preparations, was mainly follicular, with very little luteinization. The third hog preparation contained considerable amounts of LH. It is possible that this variation may be due to a difference in the physiological state of the animals being slaughtered at the time the various batches of pituitary glands were being collected, for it is well known that the pituitary glands of castrated animals contain less LH than do those of normal individuals.

The inhibiting action of sheep, hog and beef parallel their LH content, and the ratio of LH units to inhibiting units was a constant. This would lend support to the belief that the inhibiting property of pituitary extracts may be due to the luteinizing hormone.^{4, 5}

Hog and sheep pituitary glands are approximately equally active in producing ovarian enlargement. This does not mean, however, that hog and sheep glands are equal in FSH potency, but rather that those of the hog have more FSH than those of sheep. This is indicated because there is more LH present in sheep pituitary preparations than in those of hogs. Ovarian development produced by unfractionated extracts is due to the interaction of FSH and LH. Consequently more FSH must be present in hog pituitary glands with the relative small amount of LH in order to produce the same ovarian enlargement as is produced with sheep preparations, which are rich in LH. The ovaries produced with sheep preparations were always heavily luteinized while those elicited by the injection of hog substance were mainly follicular.

Beef pituitary glands produce very little ovarian development and are therefore a poor source of FSH as well as LH.

Summary. (1) It was found that sheep pituitary glands contain the greatest amount of LH, while those from cattle had very little. Hog pituitary glands showed great variation with respect to LH content but in all cases contained much less than sheep glands. (2) The inhibiting action of the pituitary preparations paralleled their LH content. (3) Hog and sheep pituitary glands are approximately equal in producing ovarian hypertrophy. Hog preparations produced mainly follicular development while those of sheep caused the development of heavily luteinized ovaries. Hog glands, therefore, contain more FSH than those of sheep. Beef pituitary glands are a very poor source of FSH. (4) The FSH and LH content of different lots of pituitary glands of the same species varies within wide limits. Nevertheless, those of each species show definite characteristics, with respect to their FSH and LH content.

11487

Effect of Three Synthetic Steroid Compounds upon Weight and Work Performance of Adrenalectomized Rats.*

DWIGHT J. INGLE. (Introduced by F. D. W. Lukens.)

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

The compound 11-desoxy-corticosterone acetate is the most active of the known steroid compounds in respect to its property of maintaining life of adrenalectomized animals. This compound (substance A) may be characterized as pregnene (4:5)-ol(21)-dione (3,20) acetate; its immediate precursor in the laboratory synthesis is (substance B) pregnene (5:6) diol (3,21) one (20) 21-mono-acetate; a third compound, (substance C) pregnene (4:5) triol (17,20, 21) one (3), was synthesized by Serini and Logemann. In substance C the stereochemical arrangement at carbon 17 is opposite to that of those steroids occurring in the adrenal cortex which also have a hydroxy group at carbon 17. In these studies substance B was found to possess definite biologic activity although to a much less extent than substance A, and substance C appeared to be inactive in the doses tested.

Male rats of the Sprague Dawley strain which weighed approximately 180 g were used in these experiments. The diet was Purina Dog Chow. Bilateral adrenalectomies were performed in one stage under ether anesthesia. The test substances were dissolved in sesame oil and administered by subcutaneous injection twice daily. The amount of sesame oil injected was kept constant at 1 cc per day for each rat. Ten animals were maintained for 7 days without treatment. Eighty-one rats were treated for 7 days. On the 7th day each animal was weighed and then subjected to the work test. The animals were anesthetized with phenobarbital sodium. The left gastrocnemius muscle was weighted with 100 g and stimulated to contract 3 times per second. Each animal received 5 cc of water twice daily by subcutaneous injection for as long as the animal continued to work. In all of the experiments stimulation was continued

^{*} I wish to express my appreciation to Dr. E. Schwenk, Schering Corporation, Bloomfield, N. J., for the samples of substance A and substance B; and to Dr. R. D. Shaner, The Organon Co., Nutley, N. J., for the sample of substance C.

¹ Serini, A., and Logemann, W., Berichte der Deutschen Chem. Gesellschaft, 1938, 71, 1362.

until the muscle ceased to respond. The details of the method have been described.2, 3

Seven days following adrenalectomy the average body-weight of

TABLE I.

			TABLE I.				
			Subst	nces			
Daily dose	preguene (4:5) ol (21) dione (3,20) acetate		pregnen diol (3,21) acet	one (20)	pregnene (4:5) triol (17,20,21) one (3)		
mg mg	Wt, g	Vork	Wt, g	Work	Wt, g	Work	
0.01	176 1	543					
		251					
		.525					
	174 1	.221					
0.03	186 3	243	153	551			
		810	172	1292			
		1971	152	1209			
	182 3	1532	167	613			
0.06	196	3743	140	dead			
	198	1929	176	1385			
	204	3283	166	882			
	198	1743	170	1410			
0.12	204	3030	178	2187			
	196	3984	162	1606			
		4152	178	2770			
	189	5956	177	2074			
0.25		5624	183	2263			
		4090	182	26			
		6695	183	2366			
	206 1	0054	196	2630			
0.50		3392	200	3910	139	16	
		2405	187	2234	158	857	
		5725	202	3085	170	830	
	191	6890	209	3096	153	1143	
1.00		8059	186	2586	137	463	
		6024	203	3464	152	1082	
		9622	220	3596	153	1467	
	200 1	0355	207	3684	178	1673	
2.00		8 194	211	3496	145	924	
		6645	204	2812	160	1781	
		3289	209	4291	165	921	
	212 1	1371	188	4490	157	625	
5.00		2019	211	3496	154	756	
		1149	204	2812			
		2806	209	4291			
	203	3935	188	4490			

² Heron, W. T., Hales, W. M., and Ingle, D. J., Am. J. Physiol., 1934, 110, 357.

³ Ingle, D. J., Am. J. Physiol., 1936, 116, 622.

the 10 untreated rats was 150 g with a range of 134-163; the amounts of work performed averaged 1276 recorder revolutions with a range of 56-2399. Each recorder revolution is equivalent to approximately 400 g-cm of work. The values for body-weight and for work of the treated animals are presented in Table I.

As evidenced by the effect of these substances upon body-weight and upon work performance, the presence of a hydroxy group instead of a keto group on carbon 3 of the pregnene nucleus decreases but does not destroy these biologic effects of the compound. This compound was reported by Waterman and co-workers to maintain the health of adrenalectomized dogs. The alteration of the molecule to the structure of substance C brought a still greater loss of activity so that substance C appeared to be biologically inactive in these tests. Earlier studies have demonstrated that although the work performance of adrenalectomized rats treated with substance A is improved over that of untreated animals, it remains very small as compared to sham operated animals. Similar values for work performance of animals treated with substance A were obtained in this study.

11488

Inhibition of Estrin-Deprivation Bleeding in Rhesus Monkey with Testosterone Derivatives Variously Administered.*

A. R. ABARBANEL. (Introduced by Carl G. Hartman.)

From Morrisania Hospital, Bronx, New York City.

Testosterone and its acetic and propionic acid esters have been shown to inhibit uterine bleeding in the castrate macaque primed with estrogens. In the present experiments a similar effect was attained with methyl-testosterone and ethinyl-testosterone (pregneninolone) and with testosterone di-propionate administered in

¹ Waterman, L., Danby, M., Gaarenstroom, J. H., Spanhoff, R. W., and Uyldert, I. E., Acta Brevia Neerlandica, 1939, 9, 75.

s Ingle, D. J., Endocrinology, 1940, 26, 472.

⁶ Ingle, D. J., Endocrinology, in press.

^{*}The writer as well as the staff of the Carnegie Laboratory of Embryology whose hospitality the writer enjoyed, acknowledges with thanks the generosity of the Ciba Corporation for the generous supply of the testosterone compounds and to E. R. Squibb and Sons for keeping the laboratory supplied with Amniotin.

¹ Hartman, C. G., PROC. Soc. Exp. Biol. and Med., 1937, 37, 87.

sesame oil. Some success also followed the oral administration of testosterone propionate given with bile salts, and of methyl and ethinyl testosterone. These experiments were carried out in the rhesus colony of the Carnegie Laboratory of Embryology, Baltimore, Maryland, in the spring of 1939.

- 1. Testosterone dipropionate. 5 mg daily injected into castrated monkey No. 584 for 16 days (June 12-27), after duly priming with estrogen (Amniotin-Squibb), inhibited bleeding and produced the usual² moderate proliferation of the endometrium, which measured up to 2.5 mm in thickness. The vaginal wall showed a fairly thick Dierks layer.
- 2. Methyl Testosterone. A. Administered parenterally. Monkey No. 596, a castrate, bled April 12, 1939 after injections of stilboestrol and was re-primed with the usual 100 R.U. of Amniotin for 9 days (April 17-25). From April 25 to May 20 incl., 5 mg of methyl testosterone in sesame oil were injected subcutaneously daily except Sunday; the animal was sacrificed on May 22. The proliferative action of the hormone was mild but bleeding was successfully inhibited.
- B. Hormone pellets placed subcutaneously. In castrated monkey No. 584, after due prinning with estrogen, eight 3 mg pellets of methyl testosterone were implanted subcutaneously at the end of the injections, April 22, 1939; 5 additional pellets on April 29. No bleeding had occurred by May 12 when biopsies were made. Results as in preceding.
- C. Hormone administered orally. Beginning on the eighteenth day of a non-ovulatory cycle, 10 mg of methyl testosterone were fed to intact monkey No. 628 to see if the hormone might extend the cycle beyond the maximum of 31 days characteristic of this animal. Feeding was continued through day 46 of the cycle and no bleeding had occurred by day 52, when endometrial biopsies were taken. While bleeding was absent, the endometrium showed almost no proliferative activity, not a single mitotic figure being seen. The organ might almost be called atrophic.
- 3. Ethinyl Testosterone (pregneninolone, anhydro-oxy-progesterone). A. Administered parenterally. Monkey No. 613, a castrate, was primed from May 16-23, 1939, with 100 R.U. of estrogen (Anniotin) daily. From May 24 to June 10 five mg of ethinyl testosterone in sesame oil were injected daily. No bleeding had occurred by June 16 when the animal was sacrificed. In some areas of the well proliferated endometrium hematomata were noted.

² Hartman, C. G., Endocrinology, 1940, 26, 449.

the 10 untreated rats was 150 g with a range of 134-163; the amounts of work performed averaged 1276 recorder revolutions with a range of 56-2399. Each recorder revolution is equivalent to approximately 400 g-cm of work. The values for body-weight and for work of the treated animals are presented in Table I.

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11489

Deacylation of N⁴-n-Acylsulfanilamides and N⁴-n-Acylsulfanilylhydroxamides in vitro.*

MATHIAS F. F. KOHL AND LAURA M. FLYNN. (Introduced by P. A. Shaffer.)

From the Departments of Biochemistry and Pharmacology, School of Medicine, Washington University, St. Louis, Mo.

The acyl derivatives of sulfanilamide are of interest because sulfanilamide is in part acetylated in the animal body before excretion, the acetylated form having only slight therapeutic activity. Higher acyl derivatives have nevertheless been shown to possess therapeutic activity comparable to sulfanilamide. Miller, Rock and Moore synthesized a series of N⁴-n-acylsulfanilamides, the therapeutic activity of which appears to increase with the length of the acyl group, up to 6 carbons, beyond which it falls off rapidly. N⁴-n-acylsulfanilylhydroxamides are found to possess therapeutic activity and high bacteriostatic value in vitro. The water solubility of these compounds decreases with lengthening of the carbon chain.

Aberhalden^{5, 6} and Bergman⁷⁻⁹ described a group of enzymes known as acylases which split acylated amino acids. More recently Michel, Bernheim and Bernheim¹⁰ have described an acylase which splits acetanilid. This enzyme, which they believe identical with the earlier described acylase, is found in high concentrations in liver and kidney of dog, cat, rabbit, ox and mouse.

We have studied the deacylation in vitro by rat liver of N⁴-n-acylsulfanilamides and the analogous N⁴-n-acylsulfanilylhydroxamides in which the NH₂-group of the sulfonamide is replaced by an

^{*} This investigation was aided by a grant to P. A. Shaffer from the Rockefeller Foundation.

¹ Harris, J. S., and Klein, J. R., Proc. Soc. Exp. Biol. and Med., 1938, 38, 78.

² Miller, E., Rock, H. J., and Moore, M. L., J. Am. Chem. Soc., 1939, 61, 1198.
³ Cooper, F. B., Gross, P., and Lewis, M., Proc. Soc. Exp. Biol. and Med., 1940, 43, 491.

⁴ Main. E. R., Shinn, L. E., and Mellon, R. R., Proc. Soc. Exp. Biol. And Med., 1940, 43, 593.

⁵ Aberhalden, E., and Ehrenwall, E., Fermentforsch., 1931, 12, 223, 376.

⁶ Aberhalden, E., and Heumann, J., Fermentforsch., 1931, 12, 572.

⁷ Bergman, M., Zervas, L., and Fruton, J. S., J. Biol. Chem., 1935, 111, 225.

⁸ Bergman, M., Zerbas, L., and Ross, W. F., J. Biol. Chem., 1935, 111, 245.

⁹ Bergman, M., and Ross, W. F., J. Biol. Chem., 1935, 111, 659.

¹⁰ Michel, H. O., Bernheim, F., and Bernheim, M. L. C., J. Pharmacol, Exp. Therap., 1937, 61, 321.

Apparently bleeding was imminent 6 days after the last injection of ethinyl testosterone. Estrogenic effects on uterus, cervix and vagina were marked.

- B. Hormone administered orally. Castrated female No. 626 was primed the usual way with estrogen. She was then given one 20 mg tablet of ethinyl testosterone by mouth daily from May 24 to June 7. She began to bleed on the 15th day after the last injection of estrogen. She was sacrificed while still bleeding; while bleeding was not prevented it was probably postponed a few days above the usual maximal interval of 10 days following moderate treatment with estrone (Amniotin). Judging from the state of the uterine, cervical and vaginal mucose the effect of oral administration proved far less than that attained by one-fourth as large a dose administered subcutaneously.
- 4. Testosterone Propionate given orally. Monkey No. 591 had her endometrium almost totally removed on Jan. 18 and on Mar. 16 she received 50 mg daily of testosterone propionate. A uterine biopsy was made on May 11. She was castrated on June 9, then fed daily for 19 days two 10 mg tablets of testosterone propionate and one 100 mg tablet of bile salts. The bleeding which usually follows castration within 10 days or less did not occur. On the other hand, the endometrium showed no signs whatsoever of proliferation, measuring but 1 mm in thickness. The condition of the vagina and the cervix, likewise, proved that a minimal quantity of the absorbed hormone reached the systemic circulation.

Summary. 1. Testosterone di-propionate prevented estrinprivea bleeding in daily parenteral doses of 5 mg in sesame oil. 2. Methyl testosterone inhibited estrin-privea bleeding when administered subcutaneously in the form of pellets or dissolved in sesame oil. Orally in daily doses of 10 mg, methyl testosterone prevented menstruation but otherwise failed to exert the slightest visible estrogenic effects. 3. Ethinyl testosterone prevented estrin-privea bleeding in the monkey when administered parenterally in doses of 5 mg a day. Given orally, it delayed slightly but did not prevent bleeding in daily doses of 20 mg, with no other estrogenic effects. 4. Testosterone propionate when administered orally in 20 mg doses along with bile salts, prevented estrin-privea bleeding, but otherwise its estrogenic effects proved minimal. 5. It is apparent that oral administration of any of the testosterone derivatives here tested is most uneconomical as compared with parenteral methods.

TABLE I.
Deacylation of Acylsulfanilamidest by Liver Suspension.

Substrate Acetylsulfanilamide Butyrylsulfanilamide	% hydrolysis			
Substrate	2 hr	5 hr	Spr	
Acetylsulfanilamide	9.1	9.1	8.1	
Butyrylsulfanilamide	14.1	27.8	30.4	
Valerylsulfanilamide	21.7	24.0	25.0	
Caproylsulfanilamide	38.3	41.0	41.9	
Heptanoylsulfanilamide	63.9	80.1	89.6	

TABLE II.
Deacylation of Acylsulfanilylhydroxamides† by Liver Suspension.

	% lıydrolysis		6.5 hr 9.6 22.7
Substrate	2 hr	4 hr	6.5 hr
Acetylsulfanilylliydroxamide	6,6	8,1	9.6
Valerylsulfanilylhydroxamide	11.5	15.9	22.7
Caproylsulfanilylhydroxamide	21.8	28.8	41.3
Heptanoylsulfaniiylhydroxamide	43.1	62.9	74.5

TABLE III.
Deacylation of Other Compounds by Liver Suspension.

	% hyd	rolysis
Substrate	4 hr	8 hr
Acetylsulfanilamide;	5.4	9.4
4:4'-Acetylaminodiphenylsulfone;	10.6	15.0
Acetanilid	58.0	72.0

[†] These acyl compounds were synthesized and presented to us through the courtesy of Sharp and Dohme, Technical Division, Glenolden, Penn.

Lewis³ in their recent study of N*-n-acylsulfanilylhydroxamides found that mice given 50 mg oral doses of the valeryl, caproyl and heptanoyl compounds showed approximately 10 mg % of diazotizable material (calculated as sulfanilamide) in the blood 2 hours later.

Conclusions. The ease of deacylation of N*-n-acylsulfanilamides and N*-n-acylsulfanilylhydroxamides in vitro by liver brei is found to increase with the length of the acyl group.

[‡] These compounds were synthesized and presented to us through the courtesy of Monsanto Chemical Company, St. Louis, Mo.

-NHOH group. On the hypothesis that a free N⁴-amino group is essential for activity it seemed important to learn the extent to which these therapeutically active acyl compounds are deacylated in the animal body. Since the analytical method available (determination of free and total sulfanilamide after hydrolysis) does not distinguish other acyl compounds from the acetyl compound appearing in urine, it seemed preferable to study the hydrolysis by tissue brei with which acetylation does not occur to confuse the results.

Methods. Extracts of liver tissue made by grinding liver with sand and an equal volume of water were strained through cheese cloth. Weights of the acyl compounds equivalent to 1 mg of unacylated compound were added to 20 cc of liver brei representing 5 g of liver in M/20 PO₄ buffer (pH 7.5). Two drops of toluene were added as a preservative. The mixtures were shaken at 37.5°C. Aliquot samples were removed at intervals for analysis. Proteins were removed with 10% trichloracetic acid (or by alcohol) and filtrates analyzed for free and total sulfanilamide by Marshall's method. Colorimetric determinations were made with an electrophotometer. Percentage of hydrolysis was calculated from the free amine found.

Comparative Hydrolysis of Sulfanilanide Derivatives. We find that the ease and rate of hydrolysis of these acyl sulfanilanide derivatives by liver brei vary with the length of the C-chain in the fatty acid. Acetyl sulfanilanide is decomposed only slowly while long acyl groups are broken off more easily. The ease of hydrolysis parallels the therapeutic activity of these compounds (reported from other laboratories^{2, 3}) which permits the view that the acyl derivatives become active after hydrolysis.

Similar experiments were performed using as substrates acetanilid and 4:4'-acetylamino diphenyl sulfone. Using the same samples of liver brei in tests with the three substrates, acetanilid consistently showed a percentage of hydrolysis higher than that of 4:4'-acetylamino diphenyl sulfone and acetylsulfanilamide.

Tables I, II and III represent the results of typical experiments with N⁴-acyl sulfanilamides, N⁴-n-acylsulfanilylhydroxamides, acetanilid and 4:4'-acetylamino diphenyl sulfone as substrates.

In vivo deacylation of acylated sulfanilamide derivatives has been shown by others from blood and urine analyses. Nitti, Bovet and Hamon¹¹ found that the formyl, acetyl, propionyl and butyryl derivatives of 4:4'-diamino diphenyl sulfone were rapidly hydrolyzed in the body to 4:4'-diamino diphenyl sulfone. Cooper, Gross and

¹¹ Nitti, F., Bovet, D., and Hamon, Y., Compt. rend. soc. biol., 1938, 128, 26.

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	% liydrolysis			
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Lewis³ in their recent study of N⁴-n-acylsulfanilylhydroxamides found that mice given 50 mg oral doses of the valeryl, caproyl and heptanoyl compounds showed approximately 10 mg % of diazotizable material (calculated as sulfanilamide) in the blood 2 hours later.

Conclusions. The ease of deacylation of N^4 -n-acylsulfanilamides and N^4 -n-acylsulfanilylhydroxamides in vitro by liver brei is found to increase with the length of the acyl group.

[†] These compounds were synthesized and presented to us through the courtesy of Monsanto Chemical Company, St. Louis, Mo.

11490

Effect of Aminophyllin, Histaminase,* and Nicotinic Acid on Histamine-Poisoned Puppy Bronchioles.;

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of Medicine, Galveston, Texas.

To the large number of drugs recommended for the relief of bronchial asthma, there have been added in the past few years two new ones; aminophyllin (theophylline with ethylene diamine) and histaminase (torantil.) Although marked clinical relief has followed the intravenous injection of aminophyllin in patients with acute bronchial asthma¹⁻³ little experimental work has been done in regard to the mechanism of action of the drug. During the course of this investigation Young and Gilbert' stated that aminophyllin greatly lessens the constricting action of histamine in the smaller bronchi and bronchioles of rabbit lung sections.

Histaminase, while less well established than aminophyllin in the treatment of bronchial asthma, is thought to inactivate any histamine implicated in acute attacks of asthma.

Nicotinic acid has been observed to benefit a few patients at John Sealy Hospital during status asthmaticus⁵ and was therefore included in this study.

Method. Bronchioles were prepared for microscopic observation by the method of Sollmann and Gilbert. Puppies were killed by the injection of air into the left ventricle. The lungs were removed and injected intratracheally with a warm solution of 10% gelatin in Ringer's solution. They were then placed in iced Ringer's solution for several hours to harden. Thin sections of lung were made free-hand with a razor, and mounted on a ring of cork in a Petri dish containing 50 cc of Ringer's solution. The dish was placed on the warm stage of a microscope, and the solution was kept at a temperature of 38°C. The size of the bronchiolar lumen was recorded by use of a camera lucida.

^{*} Histaminase was generously supplied by the Winthrop Company.

[†] A preliminary report of this work was published in Bull. John Sealy Hospital and University of Texas Medical School, 1940, 2, 55.

¹ Herrmann, G. R., and Aynesworth, M. B., J. Lab. and Clin. Med., 1937, 23, 135.

² Efron, J. Allergy, 1936, 7, 249.

³ Brown, G. T., J. Allergy, 1938, 10, 64.

⁴ Young, R. H., and Gilbert, R. P., J. Am. Med. Assn., 1940, 114, 522.

⁵ Creel, W. F., personal communication.

⁶ Sollmann, T., and Gilbert, A. J., J. Pharm. and Exp. Therap., 1937, 61, 272.

The addition of 3 mg of histamine acid phosphate usually resulted in complete closure of the bronchiolar lumen. The preparation was observed for about 10 minutes in order to rule out spontaneous relaxation, and then the test drug was added. Nicotinic acid was used in the form of sodium nicotinate, since acid per se has a dilator action.⁶

Results. Typical results with aminophyllin, histaminase, and sodium nicotinate are recorded in Table I.

			TABLE 1.			
Puppy No.	Area of norma Bronchiolar lumen, mm²	l Area after histamine	"Dilator" applied		Area after ''dilator'' drug	% of normal area
2	.474	.000	Aminophyllin	1:2000	,336	75
3	.270	.000	n ·	1:1000	.270	100
2	.456	•	**	1:1000	.558	122
5	.048	.000	Histaminase	4 units	.012	25
4	.216	.000	2.5	4 units	.000	0
4	.072	.000	Sod. nicotinat	te 1:1400	.000	0
5	091	000	7.7	1:2100	.000	n

TABLE I.

Aminophyllin 1:2000 to 1:1000 caused marked dilatation of histamine-poisoned puppy bronchioles in all 6 experiments in which it was tried. With normal bronchioles it caused moderate dilatation in 2, and had no effect in one experiment.

Histaminase caused slight dilatation of histamine-poisoned bronchioles in 3 experiments and had no effect in 2 experiments. This slight action after a few minutes was not unexpected, since one unit of histaminase is assayed to neutralize 1 mg of histamine dihydrochloride only after 24 hours' incubation at 37.5°C. Histamine was inactivated by histaminase when the pH was maintained at 7.4 by phosphate buffer during 24 hours' incubation at 37.5°C. Only by fulfilling these rigid conditions was it possible to obtain inactivation.

Nicotinic acid as sodium nicotinate 1:4200 to 1:1400 caused slight dilatation of histamine-poisoned bronchioles in two experiments and had no effect in 8 experiments.

Conclusions. Aminophyllin is an effective dilator of histaminepoisoned puppy bronchiolar sections. Histaminase and nicotinic acid had little or no effect in the concentrations used.

^{*}Histamine not applied.

11491 P

Testosterone Propionate, a Bisexual Hormone in the American Chameleon.

G. K. NOBLE AND B. GREENBERG

From the Laboratory of Experimental Biology, American Museum of Natural History, New York,

The androgen, testosterone propionate, is known to have some estrogenic effect in mammals; in reptiles this effect is greater. It enlarges the oviduct of the immature alligator and the adult lizard Sceloporus, in the latter causing a growth of the mucous glands similar to that produced by theelin. In Anolis carolinensis, it will hypertrophy both male and female genital ducts and induce both male and female sex behavior.

Pellets of crystalline testosterone propionate (Ciba)* were implanted subcutaneously into gonadectomized and intact immatures of both sexes and into similar adults. Each category was composed of 4 experimentals and 4 controls occupying the same cage. Immatures received an average of 2.68 mg, of which approximately 1.58 mg was absorbed in 24 days. Adults received an average of 5.22 mg of which 3.03 mg was absorbed in 30-36 days. A group of adult gonadectomized males and another of females were also implanted with pellets of crystalline estradiol dipropionate, averaging 8.50 mg, with absorption of approximately 1.04 mg in 17 days.

The oviduets of ovariectomized and intact immature and adult females were markedly hypertrophied by the pellets. Oviduets of adult ovariectomized controls averaged 6.79 mg while treated females averaged 36.34 mg. In cross-section, their mucosa exhibited numerous glands similar to those produced with estradiol dipropionate. Testosterone-treated immature females showed the same glandular hyperplasia of the mucosa.

Both testosterone and estradiol produced an intense keratinization of the cloaca in all treated females. Dantchakoff has described this

¹ Groome, J. R., Quart. J. Exp. Physiol., 1939, 29, 367.

² Forbes, T. R., Anat. Rec., 1938, 72, 87.

³ Gorbman, A., Proc. Soc. Exp. Biol. and Med., 1939, 42, 811.

^{*} The authors are indebted to the Ciba Pharmaceutical Products, Inc., for the testosterone propionate (Perandien) and the estradiol dipropionate utilized in this study.

[†] Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration Official Project No. 65-1-97-23 (WP. 10).

⁴ Dantchakoff, V., Compt. rend. Soc. biol., 1938, 128, 895.

effect in the Lacerta embryos of both sexes following folliculin treatment. The cloacal lining is mucoid in spayed and out-of-season Testosterone will keratinize the females and also in all males. cloacas of castrate immature and adult males. This estrogenic effect was also produced by estradiol in adult castrate males.

Testosterone propionate strikingly enlarges the ovaries of both immature and adult females. Ovaries of the immature females were as much as 3 times the size of controls. Normally only one egg enlarges in each adult ovary at one time. In treated adults, 2 or more ova developed together in one ovary. Weights of ovaries of adult controls ranged from 3.35 mg to 65.63 mg. Ovaries of treated adult females ranged from 31.29 to 309 mg. Testes, however, were smaller than in controls and showed little evidence of active spermatogenesis.

Wolffian ducts, which were very rudimentary in the immature females, were greatly hypertrophied by the testosterone pellets. They became as large as those of treated immature males. The epididymis and ductus deferens of adult castrate males were maintained by testosterone but not by estradiol dipropionate. The latter result is surprising since theelin will produce an enlargement of the male ducts in adult Eumeces and young Anolis.6

Certain tubules of the kidney were markedly hypertrophied in all testosterone-treated Anolis. This "sexual segment" of Regaud and Policard' is in secretory activity in the normal adult males and assumes this condition in all testosterone-treated males and females. Kehl's reported this effect with benzoate of androsterone in adult female Uromastix. Gonadectomized control and estradiol-treated Anolis showed uniformly small kidney tubules.

Testosterone propionate pellets will induce male courtship and copulation in immature and adult females, whether ovariectomized or intact. The same implanted females will show estrous behavior and may be copulated with by either males or treated females. Estrous behavior includes: (1) a distinctive bend of neck, and (2) voluntary submission to copulation. Testosterone-treated females were observed to stand with necks flexed in this manner before males and treated females.

Pellets of testosterone propionate increase the aggressiveness of females. One treated female eventually dominates the group and assumes the rôle of a territory-holding male. Her activity partially

⁵ Turner, C. D., Biol. Bull., 1935, 69, 143.

⁶ Clapp. M. L., Anat. Rec., 1937, 70 (Suppl. 1), 97.

⁷ Regard, C., and Policard, A., Compt. rend. Soc. biol., 1903, 55, 973.

⁵ Kehl, R., Compt. rend. Soc. biol., 1938, 127, 142.

inhibits that of the other treated females. These, however, may show male behavior and also submit to copulation. Adult males, through their larger size and aggressiveness, are able to subdue the most dominant treated female which then submits to copulation.

Testosterone propionate pellets also produce full sex activity in immature and adult castrate males. One treated immature male, on 3 separate occasions, exhibited the estrous bend of neck and was copulated with 5 times. This male likewise copulated twice in male manner.

Summary. Testosterone propionate enlarges the Müllerian duct, keratinizes the cloaca and produces estrous behavior in Anolis. It also enlarges the epididymis, ductus deferens and sexual segment of the kidney, while producing male sex behavior. It has a gonadotropic effect on the ovary but not on the testis.

11492 P

Size and Stroke of the Normal Human Heart During Neosynephrin Bradycardia.*

ANCEL KEYS AND ANTONIO VIOLANTE.

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

Marked bradycardia with pulse rates from 30 to 50 per minute is produced in normal young adults by therapeutic doses (3 to 10 mg subcutaneously) of neosynephrin—1-α-hydroxy-β-methylamino-3 hydroxy ethylbenzene hydrochloride (Keys and Violante¹). The effect persists for 30 to 60 minutes or more and is not attended by any symptoms or sensations of cardiac or respiratory embarrassment. Since repeated trials failed to disclose any significant change in the total oxygen usage during the bradycardia it seemed probable that the total minute output of the heart was not seriously diminished. If this were so there should be a very appreciable increase in the stroke output. We have investigated this question with the roentgenkymographic method of Keys and Friedell.²

^{*} This work has been supported by a Fellowship grant to the Laboratory of Physiological Hygiene of the University of Minnesota by Frederick Stearns and Co.

¹ Keys, Aneel, and Violante, Antonio, Proc. Soc. Exp. Biol. and Med., 1940,

² Keys, Aneel, and Friedell, H. L., Am. J. Physiol., 1939, 126, 741.

Trained normal young men and women subjects were used. All studies were made in the post-absorptive state in the early morning with the subject seated in a roller chair in a quiet room. When a roentgenkymographic exposure (R.K.G.) was made (66 inches), the chair was rolled into position and the subject coöperated only to the extent of holding the breath during the 1.5 second exposure. After a preliminary rest of 20 minutes or more, one or 2 R.K.G.s were made before injection of the drug. R.K.G.s were made subsequently when the bradycardia was well established—usually 10 or 15 minutes later—and when the bradycardia had begun to diminish. In some cases a final R.K.G. was made when the pulse and blood pressure were nearly normal again.

A striking alteration of the heart size was frequently apparent even from casual inspection of the resulting films. When the areas of the anterior-posterior projections were measured it was found that there is an increase of 5 to 20% or more during the period of bradycardia. The change is more remarkable when the corresponding volumes are calculated from our formula (op. cit.): vol. = 0.63 (Area)^{1.43}. To illustrate, we may cite 3 cases, selected at random, and compare the diastolic volumes in cc before and 15 minutes after subcutaneous injection of 5 mg of neosynephrin:

	Subj. E.H.	Subj. D.W.	Subj. B.N.
Before	467	503	506
After	497	601	625

The left side of the heart shows the most pronounced increase in size but all parts of the heart appear to share in the dilatation and the original form of the heart is well preserved in both systole and diastole. We have never observed any signs of pericardial restraint, in spite of the fact that the dilatation in many cases surpasses what is frequently considered to be the upper limit for immediate dilatation. In a number of cases the P.A. transverse diameter increased more than 15 mm; in one case the increase was 19 mm and in another 18 mm. In all cases the degree of inspiration was the same.

Significant increases in diastolic heart size were found in 90% of all our studies with neosynephrin. It does not appear when epinephrine or sterile saline are similarly administered. The systolic volume of the heart also increases, but to a lesser extent, so that there is a definite and usually large increase in stroke volume. In 12 studies on 8 subjects the mean stroke volume before injection was 57.5 cc; between 15 and 30 minutes after injection of neosynephrin the mean indicated stroke volume was 90.1 cc. The net effect is usually to leave the minute volume relatively constant though there may be a slight reduction in the minute volume after the largest doses

inhibits that of the other treated females. These, however, may show male behavior and also submit to copulation. Adult males, through their larger size and aggressiveness, are able to subdue the most dominant treated female which then submits to copulation.

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^{*} This work has been supported by a Fellowship grant to the Laboratory of Physiological Hygiene of the University of Minnesota by Frederick Stearns and Co.

¹ Keys, Ancel, and Violante, Antonio, Proc. Soc. Exp. Biol. and Med., 1940,

^{44, 4.} 2 Keys, Ancel, and Friedell, H. L., Am. J. Physiol., 1939, 126, 741.

Each experiment was carried out under the following conditions: Thirty to 45 minutes after breast feeding, the infant was placed in an accurately controlled air-conditioned chamber[†] and weighed by means of a Sauter scale for 30 to 90 minutes. A condition of sleep and absence of sweating were required before measurements were made.

The results are summarized in Table I.

TABLE I. Average Insensible Weight Loss at Various Humidities.

	% relative l	% relative humidity		G insensible wt loss per hr		
Case No.	Range	Avg	Infant	Kg	Sq.M.	
11	19-30	21.6	3.82	1.16	17.2	
16	31-40	35.8	3.24	.94	13.7	
<u>จี</u>	41-50	48.5	2.67	.86	12.3	
19	51-60	54.0	2.58	.80	11.7	
8	61-70	65.0	1.99	.60	8.8	
11	71-80	76.5	1.35	.39	4.7	
6	81.94	88.0	1.19	.40	5.0	

The average insensible weight loss for each 10% increase in relative humidity decreases in a straight line manner to a negligible value at 100% relative humidity. The generally accepted value for insensible weight loss of 1.0 g per kg per hour for infants falls between 25% and 35% relative humidity. This value is approximately twice that observed at 60% and 70% relative humidity. The general slope of the line indicates that for each 10% increase in relative humidity within the range studied, there is 0.46 g decrease in the hourly rate of insensible weight loss for the average newborn infant. From these data it appears that attempts to predict the energy metabolism of the newborn infant from the insensible weight loss according to the formulas of Benedict and Root for adults and Levine and Marples for infants are not valid unless the effect of the relative humidity is taken into consideration.

Summary. The rate of insensible weight loss in the unclothed, newborn male infant is decreased in a straight line manner by an increase in the relative humidity of the environmental air.

t This unit was constructed with the advice and assistance as well as the donation of special equipment from the Minneapolis Honeywell Regulator Company.

⁵ Levine, S. Z., Kelly, M., and Wilson, J. R., Am. J. Dis. Child., 1930, 39, 917.

Benedict, F. G., and Root, H. F., Arch. Int. Med., 1926, 38, 1.
 Levine, S. Z., and Marples, E., Am. J. Dis. Child., 1930, 40, 269.

(10 mg) and a net increase in minute volume frequently results from a rather small (3 to 5 mg) dose of the drug.

The contraction form shown by the R.K.G. corresponds to the RT interval of the E.C.G. in that ventricular contraction and discharge are not unduly prolonged. It is notable, however, that during much of diastole the left ventricle appears to pause at constant volume (diastasis). The R.K.G. film usually resembles extreme athletic bradycardia.

These studies are being continued with additional techniques. The first few experiments with the acetylene method have shown an increased stroke volume of the same general magnitude found with the R.K.G. method, so that apparently the dilatation does not invalidate the volume calculations.

11493 P

Effect of Relative Humidity on Insensible Weight Loss of the Newborn Infant.*

JOHN A. ANDERSON. (Introduced by Irvine McQuarrie.)
From the Department of Pediatries, University of Minnesota, Minneapolis.

Although Rubner and von Lewschew¹ and Benedict and Carpenter² noted that high relative humidity of the environmental air produced a decrease in the rate of insensible weight loss in both man and experimental animals, recent investigators³,⁴ have stated that, within the range of average environmental conditions, the relative humidity produces no significant effect. The failure of the insensible weight loss method in predicting accurately the energy metabolism of infants prompted this investigation of the effect of changes in relative humidity on the insensible weight loss. This report deals with 76 observations on the insensible weight loss in 41 unclothed newborn male infants at relative humidities ranging from 19% to 94% and at an environmental temperature of 31.2-32.5°C (87-89°F).

^{*} This study was made possible by a grant-in-aid from Mead Johnson and Company of Evansville, Indiana.

¹ Rubner, M., and von Lewschew, Arch. f. Hyg., 1897, 29, 1.

² Benediet, F. G., and Carpenter, T. M., Carnegie Inst., Washington, 1910, Pub. No. 129.

³ Levine, S. Z., Wilson, J. R., and Kelly, M., Am. J. Dis. Child., 1929, 37, 791.

⁴ Yaglou, C. P., J. A. M. A., 1937, 108, 1708.

average increase of 38% relative humidity there was an average increase in the surface temperature of the body which occurred in the following manner: the foot—2.5°C, the hand—1.5°C, the abdomen—0.8°C, and the forehead—0.7°C. Accompanying these changes, the average decrease in the rate of insensible weight loss was 1.63 g per hour.

There were two types of response of the skin temperatures of these infants. Six of the infants responded by an increase in the surface temperature of the dorsum of the hand and foot, which occurred within 10 minutes after the change from the low to the high humidity. When the high humidity was maintained at a constant level, an increase in the temperature of the abdomen and forehead occurred if the experiment was prolonged or if the increase in humidity was excessive. At this time all surface temperatures continued to increase uniformly to a level just below that of the rectal temperature, which usually remained constant. The remaining 6 infants had an increase in the surface temperatures of all parts of the body at the same time, which usually occurred within 10 minutes following the increase in relative humidity. These skin temperatures tended to stabilize at a higher level with moderate increases in humidity; or if the humidity increase was excessive, they approached the rectal temperature, which also increased slightly. Detectable sweating then occurred, followed by a fall in rectal temperature to or below the original level. The decrease of the rate of insensible weight loss was noted in both groups up to the time of the occurrence of sweating.

The increase in the surface temperature of the newborn infant induced by increasing the relative humidity of the environmental air offers an explanation of the mechanism involved in the decrease of the insensible weight loss under the same conditions. The systematic manner in which the surface temperatures of the foot, hand, abdomen, and forehead increase with the increase in relative humidity is similar to that reported by Freenian and Lengyel for adult human subjects.²

Summary. In unclothed, newborn male infants the decrease in the rate of insensible weight loss is accompanied by an increase in the surface temperature of the skin at high relative humidity. The vasomotor responses necessary for this adjustment in the heat loss mechanism under these conditions appears to be as fully developed in one-half of the infants studied as in adult subjects.

² Freeman, H., and Lengyel, B. A., J. Nutrition, 1939, 17, 43.

11494 P

Effect of Relative Humidity on Skin and Rectal Temperatures of the Newborn Infant.*

JOHN A. ANDERSON. (Introduced by Irvine McQuarrie.)
From the Department of Pediatrics, University of Minnesota, Minneapolis.

In a previous investigation on unclothed newborn, male infants the author showed that an increase in the environmental relative humidity resulted in a decrease in the rate of insensible weight loss. In an attempt to explain the physiologic processes involved in the adjustment of the heat loss mechanism of the body induced by high humidity, measurements of the surface and rectal temperatures were made under similar experimental conditions. The skin surface temperatures of the forehead, abdomen, and dorsum of the hand and foot were determined by means of copper constantin thermocouples. A pessor catheter containing a thermocouple was used for obtaining the rectal temperature. The insensible weight loss and the skin and rectal temperatures of twelve unclothed, newborn infants were determined first at a low relative humidity and, after a period of adjustment of 45 to 60 minutes, again at a higher humidity.

The results obtained are presented in Table I.

In the cases in which sweating did not occur (Cases 1-9), for an

TABLE I. Changes in Skin and Rectal Temperatures Induced by Change in Relative Humidity.

	Change							I.L.
Case No.		$\widehat{\Lambda}$ ir	Forehead	Hand	Abdomen	Foot	Rectal	ebange g
1	47	.0	.0	+1.7	+0.6	+2.4		-2.06
2	38	+ .1		+2.8	+1.1	+3.2	+.4	1.65
3	34	+ .8		+1.7	+1.2	+2.0	+.9	—1.7 2
	40	+ .3		+0.8	-1.0	+2.5	+.8	-1.35
4 5	56	+ .8	+ .9	+2.1	+1.0	+4.3		-2.34
Ğ	34	+ .3	+1.0	+2.1	+0.9	+1.6		-1.82
7	26	+ .7	•	+0.2	+1.6		.0	-0.44
8	25	+ .1		0.0	+0.2	+0.8	.0	-0.56
9	44	+2.2	+ .9	+2.6	•	+3.5	+.7	-2.76
Avg	38	+0.4	+ .7	+1.5	+0.8	+2.5	+.4	-1.63
21.48			in Which	Sweat	ing Occurre	d.		
10	24	.0	+ .1	+1.1	+0.3	.0	.0	+0.04
11	$\frac{24}{24}$	+ .2		•	.0	+2.0	+.2	+1.25
12	46	+1.0	+1.8		+1.7	+4.5	0.	+0.64

^{*} This study was made possible by a grant-in-aid from Mead Johnson and Company of Evansville, Indiana, and by the donation of special equipment, together with advice and assistance, from the Minneapolis Honeywell Regulator Company.

¹ Anderson, J. A., PROC. Soc. Exp. Biol. and Med., 1940, 44, 464.

10 to 20 microns in diameter. In transplant they develop to a considerably larger size. Four strains were isolated in pure culture and their properties will be more closely studied.

The tiny colonies were present in abundance in all cultures except one. In 2 cases they were associated with the gonococcus; in 3 they were found in the absence of gonococcus. In one case they persisted in the cervical smear even though the gonococcus disappeared following the administration of sulfanilamide.

In a previously described case a similar organism was isolated in pure culture from a suppurated Bartholin's gland.³ At that time, it was thought the patient's contact with rats might have been responsible for the infection. With the knowledge that similar organisms occur frequently in the female genitalia it seems more probable that the previously observed suppuration was caused by a human strain and that such strains are potentially pathogens. Unfortunately, the strain isolated from the suppurative lesion was lost, therefore its origin cannot be established. According to Sabin's observations, mice often harbor pleuropneumonia-like organisms in the conjunctiva.⁴ These organisms, although usually harmless, may under appropriate conditions become pathogenic. The pathogenicity of the cattle, goat and rat strains is well known. It is of special interest that all members of the pleuropneumonia group produce acute or chronic joint lesions.

At present, it is impossible to state whether the strains isolated from female genitalia are potentially pathogenic although the above mentioned single observation suggests that they may be. They may only represent another variety of the many unknown saprophytic microörganisms of the nucous membranes. The frequent presence of a member of the pleuropneumonia group of microörganisms in human beings certainly deserves further study.

³ Dienes, L., and Edsall, J., Proc. Soc. Exp. Biol. and Med., 1937, 36, 740.

⁴ Sabin, A. B., Science, 1939, 90, 18.

11495

Cultivation of Pleuropneumonia-Like Organisms from Female Genital Organs.*†

L. DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital, and the Robert W. Lovett Memorial for the Study of Crippling Disease, Harvard Medical School. Boston.

The technic formerly described for staining bacterial cultures in situ on the surface of agar was employed in studying routine plates submitted for gonococcus examination. In the course of 2 months pleuropneumonia-like organisms were demonstrated in the cervical secretions of 5 patients. The medium used for the gonococcus is similar to the medium employed formerly in cultivating Streptobacillus moniliformis and pleuropneumonia-like organisms. It is essentially a sedimented boiled blood agar to which is added 30% buffered ascitic fluid. The plates are incubated for 2 days in partial CO₂ tension.

Pleuropneumonia-like organisms were present in the genitals of about one-third of the females. Thus far similar organisms have not been found in plates inoculated with secretions from the urethra or prostate of males or from eyes of babies suspected of gonococcus infection. However, the female and male material examined was not comparable. The majority of female patients had pelvic infections, while the cultures from males were mostly release cultures from treated gonococcal patients. Women without pelvic disease were not studied.

The group of pleuropneumonia-like organisms is characterized at present by purely morphological criteria. The organisms cultivated from the female genitalia are indistinguishable in morphology and in the appearance of colonies from the strains isolated from rats and mice.² The young colonies consist of very small pleomorphic granules and filaments which grow into the medium and are stained deeply in situ with methylene blue. The surface of fully developed colonies consists of large bodies (3 to 10 microns) which are at first deeply stained but which later become vacuolized and produce a foam-like structure. After 48 hours the colonies are often only

^{*} The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

[†] This is publication Number 47 of the Lovett Memorial,

¹ Dienes, L., J. Inf. Dis., 1939, 65, 24.

² Dienes, L., and Sullivan, E. R., Proc. Soc. Exp. Biol. And Med., 1939, 41, 424.

tures. The occurrence of such organisms with the gonococcus has been indicated in a preceding note.³ The pleuropneumonia-like organism grows independently of the gonococcus, and mixed cultures are easily separated. The tiny secondary colonies develop only in connection with the large swollen bacterial forms and thus far all attempts to grow them separate from the parent organism have failed. Morphologically, the secondary growth is very similar to a young growth of the pleuropneumonia-like organism.

11497 P

Developmental Relationship Between Pars Intermedia of Pituitary and Brain in Tadpoles.

WILLIAM ETKIN. (With the assistance of Rose Lotkin.)

From the American Museum of Natural History and the College of the City of
New Fork.*

An inhibitory control of the growth and functional activity of the pars intermedia of the hypophysis of the tadpole through the infundibulum has been suggested by the author to account for the finding of overgrowth and excess activity of this gland in grafts, and after infundibular lesion.1 In his careful work with pituitary grafts in the salamander, however, Blount' reported an intensity of pigmentation only in proportion to the number of grafts and no overgrowth in the grafts. Since in Blount's work successful grafts were secured only when brain was transplanted with the gland, whereas in this author's work with tadpoles the grait took successfully independently of the presence of brain it was thought that the circumstance of the presence of brain with the primordial graft might account for the difference in the characteristic growth picture of the graft in these two cases. This theory would be consistent with the first mentioned hypothesis of inhibitory control of the p. intermedia through the infundibulum. To test this, a series of grafts of the pituitary was made with and without brain.

The experiment was performed on Rana pipiens tadpoles. The

2 Blount, R. F., J. Exp. Zool., 1932, 63, 113.

¹ Dienes, L., Peoc. Soc. Exp. Biol. and Med., 1940, 44, 465.

^{*}Assistance in the preparation of these materials was furnished by Works Projects Administration for the City of New York, Project Number 65-1-97-23 W.P. 10.

¹ Etkin, W., and Resemberg, L., Proc. Soc. Exp. Biol. and Med., 1935, 39, 332.

11496 P

L Type of Growth in Gonococcus Cultures.*;

L. DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital, and the Robert W. Lovett Memorial for the Study of Crippling Disease, Harvard Medical School, Boston.

It was described in a former note that in cultures of various Gram negative bacteria tiny secondary colonies similar in many respects to the L1 colonies of *Streptobacillus moniliformis* develop.¹ The appearance of these colonies is always preceded by the transformation of the bacteria into large swollen forms. More recently it has been observed that the secondary colonies develop from these large forms.²

A similar course of events was observed in gonococcus cultures. In certain cultures the cocci before disintegrating swell up into large deeply stained spherical bodies similar in every respect to the large bodies of Gram negative bacteria. If such cultures are kept one or two days at a temperature between 25 to 30°C, one notes below the colonies in the agar a slight secondary growth very similar to the L type of growth observed in colon bacillus and influenza colonies. It consists of small granules and fine filaments which usually degenerate in 24 hours. In gonococcus cultures this peculiar secondary growth does not develop as abundantly nor as distinctly as in the cultures of Gram negative bacteria, and without the experience obtained with the latter it would probably have been overlooked. The main evidence in support of the supposition that this slight transient growth corresponds to the development of the L type colonies is the essential similarity of the whole process in different bacterial cultures.

The observation of this process in gonococcus cultures possess some importance inasmuch as it is the first example of the occurrence of this process in a species of bacteria besides the Gram negative bacilli. Attempts to demonstrate a similar secondary growth in the colonies of Gram positive cocci, especially in the colonies of streptococci, have been unsuccessful thus far.

The process described in this note has nothing to do with the presence or absence of pleuropneumonia-like organisms in the cul-

^{*} The expenses of this investigation were defrayed in part by a grant from the Commonwealth Fund.

t This is publication Number 48 of the Lovett Memorial.

¹ Dienes, L., Proc. Soc. Exp. Biol. and Med., 1939, 42, 636.

² Dienes, L., Proc. Soc. Exp. Biol. and Med., 1940, 43, 703.

velops separated from the infundibulum its growth is excessive, it shows cellular hypertrophy and produces an excess of the pigmentary hormone which induces intense hyperpigmentation in the host. The infundibulum normally controls p. intermedia function by inhibition.

11498 P

Influence of Age on Rate of Immune Response of Mice to Formolized Equine Encephalomyelitis Virus.

ISABEL M. MORGAN. (Introduced by Peter K. Olitsky.)

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

It has been reported¹ that the ability of mice to be immunized with formolized virus of Eastern equine encephalomyelitis increases with age. The question then arose as to whether this was an expression of different rates of development of immune response, or rather of maximum responses of which various age-groups were capable. Rate of antibody response to a trypanosomal infection in rats was found to increase with age.²

The rate of development of neutralizing antibodies in serum of mice of 3 age-groups was studied. Mice 3 months, 14-15 days and 4-5 days of age were injected intraperitoneally on the 1st, 3rd and 5th days with 0.2-0.25 cc of formalin-inactivated virus of Eastern equine encephalomyelitis. This consisted of a 10% suspension of infected mouse brain in 0.5% formalin, which proved to be non-infectious on intracerebral injection of normal mice. Mice in each group were bled from the heart and the sera pooled, on the days indicated in Fig. 1, i.e., 4th, 5th (4 hours after the last injection of formolized virus), 6th day, etc. Serum-neutralizing antibodies were measured by the intraperitoneal protection test³ in normal mice from 13 to 15 days of age, using 4 mice for each virus dilution-serum mixture. Sera of different age-groups taken on the same day were compared simultaneously.

The antibody titer is recorded in Fig. 1 as doses of virus neutralized. This was calculated from the difference between the infective

¹ Morgan, I. M., Proc. Soc. Exp. Biol. and Med., 1939, 42, 501.

² Kolodny, M. H., Am. J. Hyg., 1940, 31, 1, Sec. C.

³ Olitsky, P. K., and Harford, C. G., J. Exp. Med., 1938, 68, 173.

hosts were tadpoles which had been hypophysectomized in the tailbud stage and used when about 12 mm total length. Only silver (successfully hypophysectomized) animals were used. The site for the implant was prepared by removing the eyeball through a slit in the dorsal skin, thus leaving a relatively large pocket for the reception of the graft. The grafts were taken from tail-bud embryos. In one series the pituitary primordium with as little adherent tissue as possible was used, and in the second series a variable amount of adjacent brain material was included with each graft. Normal unoperated controls were run simultaneously. When tadpoles were fully grown they were killed and the head serially sectioned.

In the first series of grafts without brain the same experimental types obtained as previously. Thus of a total of 18 surviving experimentals of this type 7 became very much darker than normal and showed on sectioning enlarged p. intermedia grafts with cellular hypertrophy and intense basophilia in the cytoplasm, 2 showed approximately normal pigmentation and on sectioning showed diffuse degenerating grafts with apparently little p. intermedia, 9 remained silver in color and the 3 of these which were sectioned showed no grafts persisting.

In the group receiving grafts with brain and pituitary 32 survived well. Of these 6 showed marked hyperpigmentation. The sections of these animals showed the characteristic hypertrophy and hyperplasia in the p. intermedia. Varying amounts of brain were present in the graft. In no case was the gland in contact with recognizable infundibular tissue though in some it was in contact with other parts of the brain.

Ten animals which fell into the silver class showed on sectioning no clear p. intermedia tissue in the graft. The critical class of experimentals was the group of 15 individuals which showed either normal pigmentation or a moderated degree of hyperpigmentation. Of these 6 proved to have degenerating grafts with doubtful or very scattered p. intermedia and 9 showed normal well formed p. intermedia grafts in which the cells were normal in appearance. Seven of these grafts were directly in contact with recognizable grafted infundibular tissue, one was directly in contact with brain tissue of uncertain form and one was in contact with the infundibulum through the intermediation of p. anterior tissue.

This experiment supports the following interpretation. When p. intermedia develops in contact with the infundibulum its growth and histological development are normal and its functional activity is not much if at all in excess of normal. When, however, it de-

The "14-days-old" mice were not uniformly resistant; 50% of mice receiving from 1 to 1,000 doses survived. The proportion surviving, of those tested 2, 3 and 4 weeks after immunization, did not increase. It was shown therefore that the high degree of active immunity of "3-months-old" mice was maintained during the 2-4 weeks' period following immunization; and the low degree of resistance of mice immunized when 14 days old did not change significantly during that period, in spite of the observation (Fig. 1) that mice of this age group possessed maximum titer of circulating antibodies.⁴

Summary. The rate of development of neutralizing antibodies in serum of mice immunized with formalin-inactivated virus of Eastern equine encephalomyelitis has been shown to increase progressively with age. The antibodies in serum of mice immunized at a very early age did not reach the maximum titer found in mice immunized

when older.

The low degree of active immunity to intracerebral injection of active virus induced in mice 14 days old at the beginning of immunization did not increase from 2 to 4 weeks after immunization. During that interval, mice immunized at 3 months of age maintained a high degree of active immunity.

11499 P

Chronic Histamine Action.*

CHARLES F. CODE AND RICHARD L. VARCO. (Introduced by M. B. Visscher)

From the Departments of Physiology and Surgery, University of Minnesota.

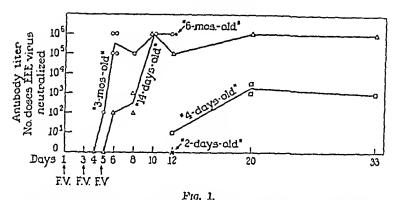
Histamine placed in the body in watery solution rapidly produces acute effects of relatively short duration (Dale and Laidlaw¹). During the past 2 years studies have been undertaken to develop a procedure by which injected histamine would act over prolonged periods and produce chronic effects. The ultimate aim of the investigation was the study of chronic histamine poisoning.

Experimental Procedure. Histamine was administered by sub-

⁴ Olitsky, P. K., and Harford, C. G., J. Exp. Med., 1938, 68, 779.

^{*}Part of the expense of this research has been defrayed by grants from the Committee on Scientific Research of the American Medical Association (No. 526 and 556) and the Graduate School of the University of Minnesota.

¹ Dale, H. H., and Laidlaw, P. P., J. Physiol., 1910, 41, 318.



Rate of development of neutralizing antibodies in serum of mice of various ages in response to 3 doses of formolized Eastern equine encephalomyelitic virus (F.V.).

titer in control and test groups of mice.* It was found that differences in titer must be more than tenfold to be significant. In serum of mice immunized at 3 months of age, neutralizing antibodies began to appear on the 5th day reaching a maximum by the 6th day. In mice 14-15 days old at the beginning of immunization, antibodies were not demonstrable until the 6th day and reached a maximum between the 8th and 10th days, which was maintained for at least 23 days longer. Considerably slower in response were mice 4-5 days old at the beginning. A minimum of antibody was demonstrable on the 12th day. Sera taken on the 20th and 33rd days protected 1/4 to 3/4 of mice in groups receiving a wide range of virus dilutions, indicating moderate antibody content. This is in contrast with sharp endpoints obtained with weak or strong antiserum. "2-days-" and "6-months-old" mice are also shown. Thus the rate of antibody production was found to increase with age. certain age, the final titer reached depended on the age at which imnunization was begun.

In order to determine whether the low grade of active immunity reported previously¹ in young mice would increase with time, 2 large groups of mice, 14 days and 3 months old, were immunized as described. Mice from each age group, as well as 3-months-old normal controls, were tested by intracerebral injection of tenfold dilutions of active virus after 2, 3 and 4 weeks. The "3-months-old" immunized mice resisted 10⁷, 10³-10⁸ and 10⁸-10⁷ doses respectively, as measured by difference in titer between control and test groups.

^{*} For example, if the titer of virus in the presence of normal serum were 10-7 and with test serum, 10-5, the difference in titer would be 102 or 100 doses neutralized.

The "14-days-old" mice were not uniformly resistant; 50% of mice receiving from 1 to 1,000 doses survived. The proportion surviving, of those tested 2, 3 and 4 weeks after immunization, did not increase. It was shown therefore that the high degree of active immunity of "3-months-old" mice was maintained during the 2-4 weeks' period following immunization; and the low degree of resistance of mice immunized when 14 days old did not change significantly during that period, in spite of the observation (Fig. 1) that mice of this age group possessed maximum titer of circulating antibodies.4

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11499 P

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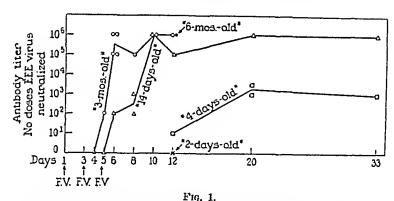
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^{*} For example, if the titer of virus in the presence of normal serum were 10-7 and with test serum, 10-5, the difference in titer would be 10² or 100 doses neutralized.

these experiments prolonged action of a single injection of histamine was obtained by suspending the histamine particles in a beeswax mixture.

11500

Salt After Adrenalectomy. I. Growth and Survival of Adrenalectomized Rats Given Various Levels of NaCl.*

EVELYN ANDERSON, MICHAEL JOSEPH AND VIRGIL HERRING. (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology and the Department of Medicine of the University of California, Berkeley and San Francisco, California.

It is well known that the administration of sodium chloride to animals deprived of their adrenals considerably delays the onset of adrenal insufficiency, but the importance of the amount of sodium chloride given such animals has not received adequate attention. There is an "optimal" amount of sodium chloride from which such an animal derives benefit; an excess of sodium chloride is injurious. Moreover, inadequate study has been given the matter of the degree to which functional restoration occurs in adrenalectomized animals given salt. These are the problems which concern us here. This study deals with the growth and survival of adrenalectomized animals in response to varying amounts of sodium chloride. Two succeeding reports deal with the urinary excretion of Na and K and with the storage of fed carbohydrate by such rats.

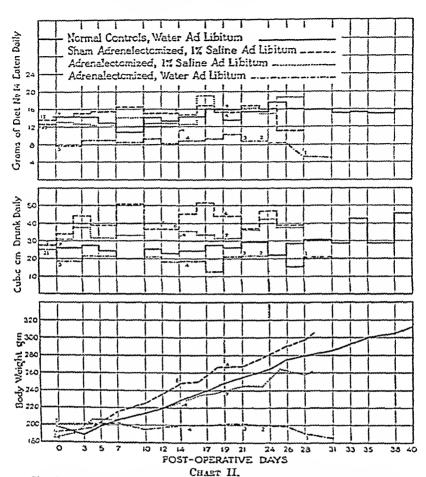
Adrenalectomized rats fed a standard diet and allowed to drink 1% NaCl take in an amount of salt which appears to be optimal for maintaining growth and health. For this study male rats were used; they were 10 weeks of age at the time of adrenalectomy, and weighed approximately 200 g. Controls were subjected to a sham operation in which the adrenals were dissected free of the surrounding tissue but not removed. From a group of 25 adrenalectomized rats given 1% NaCl solution, 15 had an average survival of 45 days (range 12-80 days) and 10 lived beyond the 110th day post-operative. During the course of the experiment all of the animals were tested for completeness of adrenalectomy by withdrawing salt and allow-

^{*}We wish to acknowledge the assistance of the Federal Works Progress Administration, Project No. OP 65-1-08-62, Unit A-5, and the Christine Breen Fund.

cutaneous or intramuscular injection. All doses mentioned are in terms of histamine base. Two tests were employed to determine the efficacy of the methods used to slow the rate of absorption of histamine from the sites of injection. The first was a comparison in normal guinea pigs of the effects of large doses of histamine in saline solution with the effects of the same dose of histamine in other substances. While this method was satisfactory for the preliminary experiments it was inadequate for the quantitative determination of the extent and degree of prolonged histamine action. The second test was the measurement of the amount and quality of the secretion from gastric pouches of dogs made according to the method of Heidenliain. This test provided a quantitative basis for studying the extended action of histamine. The pouches were prepared under ether or nembutal anaesthesia using the usual surgical technic and sufficient time for recovery was allowed before tests were commenced.

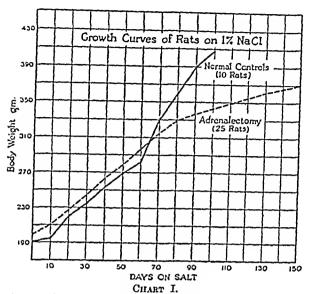
Histamine was first ground with glycol stearate and then suspended in mineral oil. In guinea pigs, injection of this material was quickly followed by typical symptoms and fatalities. Similar results were obtained when histamine particles were covered with paraffin and suspended in oil. Definite protection was obtained with a mixture of finely powdered histamine, pure beeswax or beeswax containing resin and mineral oil. Quantities of histamine which caused fatal reactions when given in saline solution produced only mild symptoms when administered with beeswax. Hot saline extraction of this dose of the beeswax mixture gave watery solutions which when injected produced profound reactions or death. Mixture of the histamine with the beeswax had not destroyed the histamine.

The histamine beeswax mixture has been quantitatively tested by the gastric secretion method in 4 dogs. Doses of histamine ranging from 15 to 60 mg were given. As a routine the volume of material injected was about one cubic centimeter and this was divided among approximately 20 intramuscular sites. Reactions were noticed in only 2 out of 10 experiments. As a rule gastric secretion commenced 10-15 minutes after the injection and continued for from 24 to over 40 hours. The total volume of juice secreted in 24 hours expressed as equivalent volume N/10 HC1 ranged from 957 to 1919 cc. In the majority of instances the maximum rate of secretion was reached during the first 4 hours following which it declined gradually. The water, chloride and hydrogen ion loss was combated by dilute saline drinking water, saline solution by vein and under the skin and by the return of gastric juice with a stomach tube. In



Showing the growth response and the food and fluid intake of adrenalectomized rats on 1% NaCl. Normal controls on tap water; total NaCl intake 601 mg daily. Sham adrenalectomized rats on 1% saline; total NaCl 1121 mg daily. Adrenalectomized rats on 1% saline; total NaCl 940 mg daily. Adrenalectomized rats on tap water; total NaCl 339 mg daily.

339 mg daily. Another group of 21 adrenalectomized rats was permitted to drink 1% NaCl ad libitum. Their total NaCl intake was 940 mg daily. One control group had been subjected to a sham adrenalectomy. This group was given 1% NaCl ad libitum. Their total NaCl intake was 1121 mg daily. The other control group consisted of normal rats which were given tap water to drink. Their NaCl intake was 601 mg daily. The adrenalectomized rats which had a total NaCl intake of 339 mg daily failed to grow and the animals died between the 14th and 31st days post-operative. The adrenalectomized rats with a higher NaCl intake, namely 940 mg, grew as



Showing the growth response of adrenalectomized rats given 1% NaCl to drink.

ing the animals to go into a state of adrenal insufficiency. The average daily intake of sodium in food and drinking water was estimated to be about 290 mg (725 mg NaCl). The growth curve of these animals is shown in Chart I. The adrenalectomized rats on 1% NaCl continued to grow at the same rate as the control animals for about 2 months; after that the growth rate was diminished. These animals were used from time to time for testing the excretion of radioactive sodium and potassium. This entailed keeping the rats on a restricted regimen of Locke's solution with glucose for 3 days at a time. A temporary loss of body weight occurred with each experiment. The electrolyte excretion of these animals is reported in another communication.¹

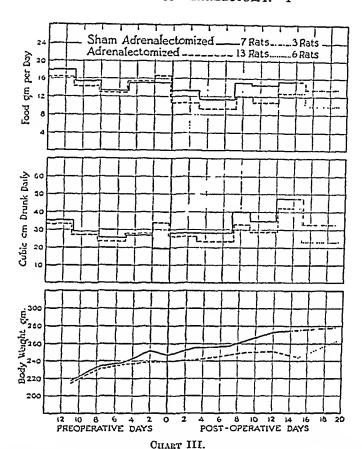
Adrenalectomized rats given 1% NaCl ad libitum consume as much food as normal rats. The intake of food and of salt solution and the gain in body weight were measured on 2 groups of adrenal-ectomized rats and on 2 control groups for a period of 30 days. These data are shown in Chart II. The stock diet given these animals contained 1.6% sodium by analysis (=4% NaCl). The animals were kept in individual cages in a chamber kept at a constant temperature of 28.5°C. One group of 5 adrenalectomized rats was given tap water to drink. Their NaCl intake in the food averaged

¹ Anderson, E., Joseph, M., and Herring, V., Proc. Soc. Exp. Biol. And Med., 1940, 44, 482.

a standard diet which contained .7% sodium by analysis (=1.75% NaCl), they grew and remained in good health for an indefinite period. They resembled the adrenalectomized rats reported above which were kept on 1% NaCl. The total NaCl intake was practically the same for both groups, averaging about 650 mg. Another group of adrenalectomized rats was given 4 cc of 5% NaCl four times a day and allowed to drink tap water ad libitum. Their NaCl intake amounted to more than one gram per day. These animals resembled untreated adrenalectomized rats; they failed to grow and their average survival period was 19.5 days (with a range of 5-28 days). When 4 cc of 5% NaCl was given 6 times a day to adrenalectomized rats, the animals were all dead within 2 days. The urinary excretion of sodium and potassium in this group of animals is reported separately.¹

The higher amounts of NaCl decreased the food intake slightly for both adrenalectomized and control rats. Food records and water intake were kept on a group of 13 adrenalectomized rats and 7 controls which were given 4 cc of 5% NaCl 4 times a day. The NaCl intake for the adrenalectomized rats was 1234 mg daily, for the controls 1352 mg (Chart III.). The difference in food intake in these two groups was slight. There was no significant growth in either group. The animals were sacrificed on the 15th and 20th days post-adrenalectomy, in order to determine the ability of these animals to store carbohydrate. These data are reported separately.

Summary. A daily intake of 650 to 940 mg NaCl for an adult adrenalectomized rat seems to be "optimal" in maintaining growth and apparent health. A daily intake of 339 mg is not sufficient for growth or survival. Large amounts of NaCl such as 1200 mg a day are injurious.



Showing the growth response and the food and water intake of adrenalectomized rats, given 16 ec of 5% NaCl daily. Sham adrenalectomized rats: total NaCl intake 1352 mg daily. Adrenalectomized rats: total NaCl intake 1234 mg daily.

well as normal animals. These animals were sacrificed on the 10th, 15th, 20th and 30th days post-adrenalectomy in order to determine the carbohydrate stores after feeding glucose. This is reported in another communication.³

In contrast to the beneficial effects of a 1% NaCl solution upon the growth and survival of adrenalectomized rats, it was found that larger amounts of NaCl in the drinking water proved to be injurious to such animals. This was tested out as follows: When adrenalectomized rats were given 4 cc of a 5% NaCl solution by stomach tube twice a day and allowed to drink tap water ad libitum and offered

² Anderson, E., Herring, V., and Joseph, M., Proc. Soc. Exp. Biol. And Med., 1940, to be published.

a standard diet which contained .7% sodium by analysis (=1.75% NaCl), they grew and remained in good health for an indefinite period. They resembled the adrenalectomized rats reported above which were kept on 1% NaCl. The total NaCl intake was practically the same for both groups, averaging about 650 mg. Another group of adrenalectomized rats was given 4 cc of 5% NaCl four times a day and allowed to drink tap water ad libitum. Their NaCl intake amounted to more than one gram per day. These animals resembled untreated adrenalectomized rats; they failed to grow and their average survival period was 19.5 days (with a range of 5-28 days). When 4 cc of 5% NaCl was given 6 times a day to adrenalectomized rats, the animals were all dead within 2 days. The urinary excretion of sodium and potassium in this group of animals is reported separately.¹

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11501

Salt After Adrenalectomy. II. Urinary Excretion of Radioactive Na and K in Adrenalectomized Rats Given Various Levels of Salt.*

EVELYN ANDERSON, MICHAEL JOSEPH AND VIRGIL HERRING. (Introduced by Herbert M. Evans.)

From the Institute of Experimental Biology and the Department of Medicine of the University of California, Berkeley and San Francisco, California.

In an earlier communication¹ we reported the use of radioactive sodium and potassium in the detection of changes in the urinary excretion rate of sodium and potassium after adrenalectomy. It was found that adrenalectomized rats fed one of our stock diets which contained 1.75% NaCl, and given tap water to drink showed an increased rate of excretion of administered radioactive sodium and a diminished rate of excretion of radioactive potassium. The rate of excretion of these tagged electrolytes could be correlated with the excretion of body sodium and potassium. We also showed that the giving of a one percent sodium chloride solution to adrenal-ectomized rats instead of tap water corrected the wastage of sodium and the retention of potassium, so that these animals excreted these electrolytes in the same proportions as normal animals.

In the preceding communication² the growth and survival of adrenalectomized rats given 1% NaCl solution has been described. Out of a group of 25 adrenalectomized rats which were given 1% NaCl to drink and which were fed a diet which contained 1.75% NaCl, 10 lived beyond the 110th day after operation. The capacity of these animals to excrete given amounts of radioactive sodium and potassium was measured from time to time.

The methods used and the standardization of the conditions necessary for this study have been described previously. The data of this study are given in Table I. It will be seen that adrenalectomized rats on 1% NaCl (with a total NaCl intake of 725 mg daily) at first excrete the administered radioactive sodium like the control animals but later show sodium retention. This discrepancy

1940, 44, 477.

^{*}We wish to acknowledge the assistance of the Federal Works Progress Administration, Project No. OP 65-1-08-62, Unit A-5, and the Christine Breon Fund. We wish to thank Dr. John H. Lawrence, of the Radiation Laboratory of this University, for supplying us with the radioactive sodium and potassium.

¹ Anderson, E., and Joseph M., Proc. Soc. Exp. Biol. and Med., 1939, 40, 347.
2 Anderson, E., Joseph, M., and Herring, V., Proc. Soc. Exp. Biol. and Med.,

II

TABLE I. Urinary Exerction of Nn and K in Adrennlectonized Rats Given 1% NaCl.

		4	Nn24		Total			1542			Total
Days post.	1315	2611	1108	120th	80th	Sth	16th	48th	11100	13611	90th
Group I Normal rats on 1% NuCl	36.7(5)* (33.0. 40.0)+	35.0(10) (30.8- 42.1)	31.9(3) (26.8- 35.8)	31.9(3) (26.8- 35.8)	81(3) (77. 87)	10(5) (8.8. 11.6)	9.7(5) (8.4. 10.4)	9.0(10) (8.2. 10.4)	11.9(3) (10.2. 12.8)	11.9(3) (10,9. 12.8)	43(3) (40.
Group 11 Adremal eto- 35.7(5) 32.3(6) 2: mixed raty (33.0. (28.6- (2.0.0)) on 15.0 NuCl 37.5) 40.0)	35.7(5) (33.0- 37.5)	32,3(6) (28,6- 40,0)	24.6(2) (23.9- 25.2)	17.8(5) (15.1. 21.5)	70(2) (63- 68)	9.6(5) (7.8- 10.0)	9.0(5) (8.4: 10.0)	10.8(6) (8.2. 12.1)	12.3(5) (9.3. 15.5)	12,6(5) (10,1. 14.3)	46(5) (37- 52)
Number of Range of va	unimals in lues.	parenthese	i,		}						

11501

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The methods used and the standardization of the conditions necessary for this study have been described previously. The data of this study are given in Table I. It will be seen that adrenalectomized rats on 1% NaCl (with a total NaCl intake of 725 mg daily) at first excrete the administered radioactive sodium like the control animals but later show sodium retention. This discrepancy

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2 Anderson, E., Joseph, M., and Herring, V., Proc. Soc. Exp. Biol. and Med.,

^{1940, 44, 477.}

Summary. The administration of sodium chloride to adult adrenalectomized rats in amounts varying from 650 mg to 1 g daily prevents the urinary sodium wastage and potassium retention which characterizes adrenalectomized animals.

11502 P

Oxidation of Tyrosine by Ultraviolet Light in its Relation to Human Pigmentation.

STEPHEN ROTHMAN. (Introduced by G. F. Dick.)

From the Department of Medicine, Section of Dermatology, and the Department of Pharmacology, University of Chicago.

In the skin of mammals tyrosinase never has been conclusively demonstrated. It has been assumed that the immediate precursor of melanin in mammalian skin is 3-4 dihydroxy-phenylalanin ("dopa") which becomes oxidized to melanin by an intracellular specific oxidase present only in normal functioning melanoblasts. The question has remained unsettled from where this dopa may originate; whether it is formed from tyrosine in the blood or in the skin.

Arnow demonstrated the formation of dopa by exposure of tyrosine solutions to ultraviolet radiation. As shown in our laboratory, this process needs a strikingly long irradiation time, namely 8-30 times as much as necessary for slightest pigmentation of human skin. In the presence of ferrous salts, however, the formation of dopa from tyrosine by ultraviolet irradiation is accelerated to such a degree that it may serve as a model of the biologic formation of dopa in human skin.

Samples containing mixtures of tyrosine and ferrous salts, irradiated with 1-3 "threshold erythema doses" yield measurable amounts of dopa but no melanin. When such irradiated samples are kept in the dark, progressively increasing amounts of precipitated melanin are formed after 16-24 hours. In this way the latent period of pigment formation in human skin is simulated by the *in vitro* experiments.

The late formation of melanin occurs for the greatest part at the

¹ Bloch, Br., Jadassohn's Handb. d. Haut. u. Geschlie. 1927, 1, 434.

² Rothman, S., Z. f. d. ges. exp. Med., 1923, 36, 398.

³ Arnow, L. E., J. Biol. Cheri., 1937, 120, 151.

increases as the period after adrenalectomy increases. This is in marked contrast to the behavior of untreated adrenalectomized animals, in which there is an increased excretion of sodium. The excretion of radioactive potassium in the adrenalectomized animals of this group was essentially the same as that of the control animals; as stated above the untreated adrenalectomized animal excretes a diminished amount of potassium. It can be noted that the excretion of body sodium and potassium bears a definite correlation to the excretion of radioactive sodium and potassium.

Increasing the amount of NaCl administered to adrenalectomized rats beyond an optimal level, proved to be injurious as described in the preceding communication.² However, these high doses of NaCl enabled the adrenalectomized rat to continue to excrete radioactive sodium and potassium like normal controls. These data are given in Table II. The animals in Group I of Table II received

TABLE II.
Urinary Exerction of Na and K in Adrenalectomized Rats on High NaCl Intake.

	Soc	lium		ıssium K42	
Days post-adrenalectomy	Na24 20th %	Total Na 20th mg	12th %	24th %	Total K 24th mg
Group I (4 ce 5% NaCl 2: daily = 400 mg	r				
Adrenalectomized rats	36.2(5)* (33.0-40.0)†	_	8.6(5) (7.8-8.8)	9.2(5) $(8.4-10.4)$	
Normal rats	35.0(10) (30.8-42.1)	_	9.7(5) (8.4-11.5)		
Group II (4 cc 5% NaCl daily = 800 mg)	4x				
Adrenalectomized rats	31.6(5) (27.2-37.5)	89(5) (85-94)	8.9(5) (8.8-10.0)	10.0(3) (9.5-10.4)	39(3) (36-43)
Normal rats	36.0(3) (33.0-37.5)	83(3) (79-88)	7.7(3) (6.4-8.8)	9.4(3) (9.1-9.5)	40(3) (36-43)

^{*}Animals per group in parentheses. †Rango of values.

400 mg NaCl administered in a 5% NaCl solution, in addition to about 245 mg of NaCl in the food. As described previously, these animals continued to grow and remained in a healthy condition. Those of Group II received 800 mg of NaCl in addition to approximately 245 mg of NaCl in the food. The adrenalectomized animals of this group were all dead by the 28th post-operative day. However, in both groups of adrenalectomized rats the excretion of electrolytes resembled that of normal rats. The total sodium and potassium excreted showed a definite correlation with the percent of radioactive sodium and potassium excreted.

11503 P

Free and Combined Sulfanilamide in Material Drained from the Human Biliary Tract.

ROGER S. HUBBARD AND RICHMOND K. ANDERSON.

From the University of Buffalo School of Medicine and the Edward J. Meyer Memorial Hospital, Buffalo, N. Y.

The concentrations of free and of combined (presumably acetyl) sulfanilamide have been determined in the material collected through T-tubes inserted in the bile ducts of human subjects after operations upon the biliary system. Bile was clarified by a mixture of trichloracetic and phosphotungstic acids. Free sulfanilamide was determined by the method of Marshall and Litchfield' after the addition of acetone to the filtrate from the pigments etc. removed by this procedure. In the determination of combined sulfanilamide, the filtrate was treated with hydrochloric acid and hydrolized for an hour in a boiling water bath before the diazo reaction was carried out. Both free and acetyl sulfanilamide* were added to the bile and were recovered quantitatively by this procedure, but the method of precipitating could not be considered as wholly satisfactory, for a trace of pigment (apparently biliverdin) was present after treatment in a fair number of specimens from some of the patients studied. The method of Marshall and Litchfield was also used in the study of urine specimens collected simultaneously with the bile from a number of the natients. Blood analyses were carried out by the technique of Bratton and Marshall.2

Although the clarification of some of the bile specimens was not satisfactory the results of 10 studies upon 5 patients agreed closely together, and justify the following statements. There was no compound in the clarified bile which gave a reaction with the diazo technique used. Shortly after one to 2 g of sulfanilamide were given by mouth, the free drug appeared in the bile draining from the T-tube, but regularly in a concentration lower than that present in the blood. This finding differs from results previously reported upon human bile from the gall bladder³ for the concentration of free sulfanilamide in such material is frequently higher than it is

¹ Marshall, E. K., Jr., and Litchfield, J. T., Science, 1938, 88, 85.

^{*} The acetyl sulfanilamide used was a synthetic product furnished through the courtesy of the National Aniline and Chemical Company.

² Bratton, A. C., and Marshall, E. K., Jr., J. Biol. Chem., 1939, 128, 537.

³ Bettman, R., and Spier, E., Proc. Soc. Exp. Biol. AND MED., 1939, 41, 463.

expense of dopa which has been formed during the radiation. However, in a few instances a slight decrease of tyrosine and a transitory increase of the dopa concentration could be observed within 1-2 hours after irradiation. This "after effect" of irradiation may be due to formation of rather stable peroxides by ultraviolet rays.

In tyrosine-ferrous salt mixtures the dopa concentration increases with continued irradiation to a certain maximum. If 50 mg % tyrosine solutions are irradiated this maximum amounts to about 5 mg %. Later, in spite of continued irradiation, the dopa concentration remains unchanged because dopa formation and oxidation of dopa to melanin keep balance with each other. Or, the dopa concentration decreases because more melanin than dopa is formed.

In long-lasting irradiation experiments it was found that in spite of a steady decrease of the tyrosine concentration the amount of melanin produced does not exceed a certain maximum. This is due to a decomposition of melanin into lighter colored, soluble products by the continued irradiation.

For clinical actinotherapy this finding indicates that after maximal pigmentation has been reached, continued irradiation does not produce a static but a dynamic equilibrium in which pigment formation and decomposition are kept in balance. Some clinicians have claimed that ultraviolet light treatment should be administered intermittently in order to assure an optimum therapeutic effect by "depigmentation periods." The experimental results, however, seem to obviate the necessity of such a procedure, and it appears justified to continue sunshine treatment in patients who already are tanned to a maximum, because biologic action of the rays continues.

Ultraviolet radiation acts on tyrosine in presence of ferrous salts similarly to tyrosinase in every detail. This fact supports the assumption that in mammals too, in which tyrosinase is not present, the primary precursor of melanin is tyrosine. The formation of dopa may occur in the skin by the action of ultraviolet rays if nonspecific oxidation catalysts are present.

Summary. The oxidation of tyrosine to melanin by exposure to ultraviolet light in presence of ferrous salts serves as a model of pigment formation by sunshine in human skin. Continued irradiation of tyrosine-ferrous salt mixtures leads to a dynamic equilibrium in which formation and decomposition of melanin are kept in balance.

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TABLE I.

Concentration of Sulfanilamide in Urine, Blood and Drainage from the Biliary

Tract.

Per	iod		Fh	uid dra	ined f	rom bi	liary (ract	Bl	0001	U	rinc
4 hr	each			pr. 6		or. 11		r. 14		r. 11 r. 14	_	r. 14
		Cor	icentra	ation o	f sulf.	anilam	ider	ng per	100 c	e.		
			Free	Total	Freo	Total	Free	Total	Frec	Total	Free	Total
Befo	rc		_		0.00							
		2 g (XXX g	rains)	of sul	fanilar	nide e	iven b	v mou	th.		
1st a	fter		0.76	0.70	1.60	1.66	1.77	1.75		3.70* 4.70†	14.3	23.6
2nd	* *		1.35	1.77	1.72	2.11	0.40	0.421		2000	30.8	45.2
3rd	,,		1.46	1.68	_	_	1.45	1.84			27.6	29.7
		2 g (XXX gr	rains)	of sul	fanila	nide g	riven b	y mou	th.		
4tlı	"		_	_	-		1.58	1.74	1.80	2.50*	20.8	37.7
5th	7 1						1.92	2.54			19.0	29.9
6th	2.7				_	-	2.37	2.96			18.1	22.3
7th	13		-				2.56	3.20			26.6	32.3

*Blood collected April 11, 1940

†Blood collected April 14, 1940.

About 5 ee of very thin bile drained from the tube during this 4-hour period. The time of the 4-hour periods of collection (before, 1st after, etc.) is calculated from time when the first dose of sulfanilamide was administered.

The blood specimens were collected at the mid-points of the 4-hour periods

against which they are recorded.

in the blood. Later, the free sulfanilamide in our experiments approximated more nearly the concentration in the blood, as would be expected from the water content of the two fluids' but the relationship was not directly proportioned to the water content. Sulfanilamide could be demonstrated in the bile 24 hours after 2 g had been taken by mouth.

During the first 4 hours after the administration of such single doses as were used in these experiments, no demonstrable amount of combined form of the drug could be demonstrated in the bile, although both free and combined forms were found in the blood during this period. After 4 hours, both compounds were present, although the concentrations of the combined forms were small, for only 10 to 20% of the total sulfanilamide occurred as the combined derivative at periods when analyses of blood and urine showed that approximately half of the total sulfanilamide reacted with the diazo reagents only after acid hydrolysis. If acetyl sulfanilamide is formed in the liver, as current experimental work indicates it is apparently not discharged in large amounts directly into the bile.

⁴ Marshall, E. K., and Cutting, W. C., J. Am. Med. Assn., 1937, 108, 953; J. Pharm. Exp. Therap., 1937, 61, 196.

⁵ Harris, J. S., and Klein, J. R., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 781; Klein, J. R., and Harris, J. S., J. Biol. Chem., 1938, 124, 613.

The findings described above, except the presence in the bile for 24 hours of appreciable amounts of free and combined sulfanilamide, are illustrated in the results of these experiments upon one subject shown in the table. As already stated, these results are essentially similar to studies made upon 4 other subjects. The experiment presented also shows that when a second dose of sulfanilamide was ingested at a time when the drainage from the biliary tract contained the drug in both the free and the combined forms, the increase in the amount of the total compound was largely or wholly in the free state. In the urine on the other hand, the larger proportion of the increase was the combined (acetyl) derivative.

11504

Occurrence of Precipitation Zones in Mixtures of Serum and Sodium Desoxycholate; Significance in Pneumococcolysis.*

Samuel Charles Bukantz,† Paul F. de Gara‡ and Jesse G. M. Bullowa.

From the Medical Service, Harlem Hospital, Department of Hospitals, New York City, and the Littauer Pneumonia Research Fund, New York University College of Medicine.

Observations by Ransom,¹ Bayer,² Sellards,³ Ponder,⁴ and Williams⁵ have shown that the lysis of red cells by bile salts is inhibited in the presence of serum. Wieland,⁶ and Donnelly and Mitchell⁷ have subjected this fixation of bile salts by serum to quantitative study. The latter felt that the bile salt-serum reaction, which manifested the Danysz effect, was one of adsorption rather

^{*} This study received additional financial support from the Metropolitan Life Insurance Company, and from Mr. Bernard M. Baruch, Mr. Bernard M. Baruch, Jr., Miss Belle N. Baruch, and Mrs. H. Robert Samstag.

[†] Littauer Fellow in Pneumonia Research.

Dazian Fellow.

¹ Ransom, F., Deutsche Med. Wochenschr., 1901, 27, 194.

² Bayer, G., (a) Biochem. Z., 1907, 5, 368; (b) idem., 1908, 9, 58.

³ Sellards, A. W., J. H. Hosp. Bull., 1903, 19, 268.

⁴ Ponder, E., Proc. Roy. Soc., B., 1922, 03, 86.

⁵ Williams, J. W., PROC. Soc. Exp. Biol. AND Med., 1932, 20, 916, 918.

⁶ Wieland, H., Naunyn-Schmiedeberg, Arch. Pharm. und exp. Path., 1920, 86, 79, 92.

⁷ Donnelly, J. L., and Mitchell, A. G., Am. J. Physiol., 1927, 79, 297.

1 ml of 4% serum broth culture plus 1 ml of various concentrations of sodium desoxycholate, in plain broth. Lysis of an 18-hour Culture of Pneumococcus III. TABLE I.

Broth Control	+++
1:10,000	+++
1:5,000	+ + + + + + + + + + + + + + + + + + + +
1:2,500	+ + + + + + + + + + + + + + + + + + + +
1:1,500	+++
1:1,250	+ +
1:1,000	++++++++++++++++++++++++++++++++++++++
1:750	++++
1:500	+ + +++ 666
1:250	‡00
	una Immed. oth 30' lture 60' Key to all tables:
37°C	Serum Broth Culture Key to

Key to all tables:

O = Clear.

T+, T++, T+++ = Various degrees of turbidity.

If = Fine flocculation.

Immed. = Immedia

than of chemical combination. They demonstrated that the reaction quantitatively followed the adsorption equation, $\frac{X^n}{-} = K$. This, however, according to Sobotka,8 is not appreciably different from the earlier mass action formulation of Wieland, $\frac{ab}{(A-a)^2} = K$. Carlinfante,9 has recently reported the occurrence of turbidity in mixtures of 5% human serum and diluted ox bile. In his experience, turbidity did not occur with dilutions of bile greater than 1 to 40.

During the course of experiments dealing with the quantitative determination of pneumococcal capsular polysaccharide in lysates, we noted certain irregularities in the clearing of 4% serum broth cultures (Pn. III) by solutions of sodium desoxycholate. Portions of this culture had been diluted 1 to 2 with broth containing the desired concentration of sodium desoxycholate, so that the final mixture contained horse serum in a 2% concentration. Under these conditions cultures containing concentrations of desoxycholate ranging between approximately 1 and 2 mg per ml failed to clear after several hours at 37° C and overnight refrigeration while with several higher and lower concentrations the same cultures cleared completely in one half hour at 37° C. (Table I). Smears and cultures from the turbid fluids containing 1 to 2 mg per ml of desoxycholate, however, failed to reveal bacteria.

The reactions were, therefore, repeated with plain as well as serum broth cultures. The middle zone of turbidity did not develop with plain broth cultures nor did control tubes of plain broth, without organisms, become turbid in the presence of those concentrations of desoxycholate employed in the lysis studies. The pneumococci grown in serum-broth were washed 3 times in saline and one-half the sediment resuspended in plain saline and the other half in 4% serum saline. These were also diluted 1 to 2 with broth containing various concentrations of sodium desoxycholate. The plain saline suspension cleared completely with concentrations of sodium desoxycholate up to and including 1-2500; the serum saline suspension became turbid in the same zone encountered with the unwashed serum broth cultures. Control tubes with a final concentration of 2% serum became turbid in the identical zone. (Table II).

We employed the technique of Donnelly and Mitchell,3 mixing varying concentrations of sodium desoxycholate with 2% horse

s Sobotka, H., Physiological Chemistry of the Bile, Williams and Wilkins, Baltimore, 1937, page 131.

² Carlinfante, E., Sperimentale, Arch. di Biol., 1933, 92, 8.

TABLE II. Lysis of 18-hour Plain Broth Culture of Pucumococcus III and of Washed Scrum Broth Culture Resuspended in Saline with or without

Serum.

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	1			+	H:	 	T+++	++	-	0 0	Ö	I (determl)	
	ı	- 1			+++1	 			٠ ح) []	Ö	iu Table	
ychelate.		ı	+ + 50	5	۲۵ +	Ö	+++4	+	Ö	0	5	described	
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		Imme	ος ΟΔ		30, 0V		n aline OV	I Immo	sms 30'	0	y of this		
200	37.0	Plain broth*		Washed sedi-	ment suspensio in salino	Washed sedi-	ment suspensio in 4% serum s	4% serum brot	without organia		tometer).	•	
	Section 1 Desoxycholate.	1:250 1:500 1:750 1:1.000 1:1 ogn 1:1:00	1:250 1:500 1:750 1:1,000 1:1,250 1:1,500 1:2,500 1:5,000 1:10,000	1:250 1:500 1:750 1:1,000 1:1,250 1:2,500 1:5,000 1:10,000 Control of Control	11:250 1:750 1:1,000 1:1,250 1:1,500 1:10,000 Dilue 30° C<	1:250 1:500 1:750 1:1,000 1:1,250 1:2,500 1:5,000 1:10,000 Cont. 30,	Institute of the control of the cont	1:250 1:500 1:750 1:1,000 1:1,500 1:2,500 1:5,000 1:10,000 Control No. C C C C C C C C C C C C C C C C C C C	1:250 1:500 1:750 1:1,000 1:1,250 1:1,500 1:2,500 1:5,000 1:10,000 Gontrol T+ T	1:250 1:500 1:750 1:1,000 1:1,500 1:2,500 1:5,000 1:10,000 Control No. C C C C C C C C C C C C C C C C C C C	Stock	Stock 1:250 1:500 1:1,500 1:1,500 1:2,500 1:2,500 1:1,000 Diluent	1:250 1:500 1:750 1:1,000 1:1,250 1:1,500 1:2,500 1:10,000 Gontrolled

Final Concentration of			
TABLE III.	nonne and Mornial Adiso Science	Liorse Serum 2% in All Tubes.	Final Concentration Sodium Desoxycholate.

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Mg per ml.				plain] experi
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-		2.4	+++++	dissolved directly on before use in
		3.0	00000000	cholate, C.P., was diss
		3.4	00000000	xycholate, n 1% alco
		4.0 (1:250)	00000000	odium deso
		At room temperature	100 100 100 100 100	Powdered sodium desoxycholate, C.P., was dissolved directly in plain broth for use in experiments recorded in Tables I and II.

TABLE II. Lysis of 18-hour Plain Broth Culture of Pueumococcus III and of Washed Serum Broth Culture Resuspended in Saline with or without Serum.

(Final concentration of serum-2%.)

				Final Dil	Final Dilution of Sodium Desoxycholate.	m Desoxye	holate.				
37°C		1:250	1:500	1:750	1:1,000	1:1,250	1:1,500	1:2,500	1:5,000	1:10,000	Diluent Control
Plain broth*	Immed. 30' OV	+00	H00	+00	+00	++55	+++00	+++00	++++	+++	+++ +++ +++
Washed sedi- ment suspension in saline	Immed. 30' OV	+ +00 +	#20	÷00	F00	#00 +	#00	+ + +++ 666		• +++	• +++
Washed sedimed. ment suspension 30' in 4% serum saline OV	Immed. 30' line OV	000	++++	+ +++ 445	4 4 4 +	+ 000	++++		+++	+++	• ++-
4% serum broth Immed. without organisms 30%	Immed. ns 30' OV	000	++ ++ 440	++ ++ 0HE	00H	000	000		- - - - - -	+ + + 000	+ + +
"The density tometer).	of this et	ılture was	s adjusted to	o equal tha	"The density of this culture was adjusted to equal that of the 18-hour culture described in Table I (determined by photronreflee-	r culture	described	in Table	I (determi	ned by ph	otronreflee-

flicting opinions regarding the influence of serum on the lysis of pneumococci by bile salts. 11, 15, 16

Summary. Turbidity and flocculation developed in 2% horse serum broth containing concentrations of sodium desoxycholate between 1.2 and 2.4 mg per ml at a pH of 7.3 and cleared on the addition of an excess of sodium desoxycholate. Apparent failures of lysis of serum broth cultures of pneumococci were due to the occurrence of such turbidity because no bacteria were found in these mixtures.

11505

Influence of Arsenicals, Bismuth and Iron on the Plasma Ascorbic Acid Level.

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From the Departments of Chemistry and Pediatrics, Northwestern University

Medical School, Chicago, the U. S. Public Health Service, and the Department of

Health, Chicago.

Several reports indicate that the poisonous effects of a number of drugs like benzene, lead.¹ phenylcinchoninic acid.² and glycerol³ and especially the arsenicals¹ may be counteracted successfully by giving suitable doses of ascorbic acid.

From examination of the urine Dainow' concluded that patients who showed symptoms of intolerance to arsenicals were in a state of hypovitaminosis C. By administration of ascorbic acid, these hypersensitive patients became able to tolerate neoarsphenamine. Other investigators reporting similar observations, emphasize

¹⁵ Boecker, E., and Kauffmann, F., Bakteriologische Diagnostik, 1st Ed., 1931, Berlin, J. Springer, p. 71.

¹⁶ Park, W. H., and Williams, A., Pathogenic Microörganisms, 10th Ed., 1933, Lea and Febiger, Phila., p. 353.

Abt. A. F., and Farmer, Chester J., The Vitamins, Chapter XXII, American Medical Association, Chicago, 1939.

² Bertellotti, L., Mineria, Medic., 1939, 30, 254.

³ Pfeiffer, C., and Arnove, T., PROC. Soc. Exp. Biol. and Med., 1937, 37, 467.

⁴ Dainbow, T., Presse med., 1937, 45, 1679; Annal. Dermat. et Syphil., 1939, 10, 139.

⁵ Landrisch, S., Polska gas. lek., 1937, 16, 575; J. A. M. A., 1937, 109, 834.

⁶ Cormia, F. E., Canad. Med. Assa. J., 1937, 36, 392.

⁷ Monte-ano quoted by Bertellotti.2

⁵ Biss quoted by Dainow.4

⁹ Tibor, S., Ortosi Hetelap, 1939, 83, 811.

¹⁰ Santiago, A., Zbl. Hast und Geschl. Krlh., 1938, 60, 74,

¹¹ Takahashi quoted by Bertellotti.2

serum broth to a constant volume of 5 ml. Table III shows the development of turbidity and flocculation in these mixtures and in the control tubes. Turbid fluids were not cleared by centrifugation at 2,000 rpm for 20 minutes nor by boiling. Addition of 3% acetic acid precipitated the bile salt. Normal NaOH in excess

cleared the turbid mixture.

It will be noted in Table III that a 2% horse serum broth remained clear in the presence of 4 mg per ml of sodium desoxycholate while a tube containing 2 mg per ml became turbid promptly and had developed fine flocculation after standing overnight. To 5 ml of the turbid solution containing 2 mg per ml of the bile salt an additional 10 mg of dry sodium desoxycholate was added. There was prompt and complete clearing of the solution. The addition of an excess of serum to another turbid fluid failed to clear the mixture. The pH of the solutions containing 2 and 4 mg per ml of sodium desoxycholate, respectively, was determined with the quinhydrone electrode and both were found to be at 7.3. An alteration of pH was apparently not the factor responsible for the clearing.

We have also observed that solutions of sodium desoxycholate of greater than 2.0 mg per ml concentration, either in broth or in phosphate buffer near neutrality, gelled after standing at room temperature. This gelation occurs in the absence of serum and, as was also noted by Schaub and Reid, disappears on warming. Preliminary observations apparently indicate that gelation is more rapid and complete at neutrality than it is on the alkaline side of neutrality. The occurrence of this gel in cultures containing bile salts at a pH of 6.8 has been previously described by Mair and is an evidence of their colloidal character of which others have been described. Later 1 and 1 an

The present observations indicate that serum interferes with the interpretation of lysis in the presence of certain concentrations of sodium desoxycholate. However, several text-books offer con-

[§] The reactions were unaffected when a Sörensen phosphate buffer at a pH of 7.3 was substituted for peptone broth as diluent. Stock powdered sodium desoxycholate did not dissolve in broth until heated to 65°C. After evaporation from alcohol, however, rapid solution took place at room temperature. The pH of the mixture of serum with the highest concentration of desoxycholate employed was 7.3, determined with the quinhydrone electrode.

¹⁰ Schaub, I. G., and Reid, R. D., J. A. M. A., 1938, 111, 1285.

¹¹ Mair, W., in A System of Bacteriology, Med. Res. Council, 1929, London, Vol. II, page 168.

¹² Achard, C., Boutaric, A., and Berthier, P., Compt. Rend. Acad. Sci. (Paris), 1937, 204, 1049.

¹³ Bashour, J. T., and Bauman, L., J. Biol. Chem., 1937, 121, 1.

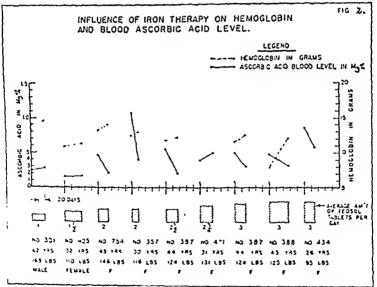
¹⁴ Roepke, R. R., and Mason, H. L., J. Biol. Chem., 1940, 133, 103.

or above). When showing severe symptoms of intolerance, a decline of the plasma level occurred in spite of the oral administration of ascorbic acid during treatment. It was frequently observed that a marked lowering of the plasma level followed the administration of neoarsphenamine in patients showing no intolerance to the drug (Fig. 1).

When bismuth was given in doses routinely used for antiluetic treatment, no appreciable effect was observed either on the plasma ascorbic acid or hemoglobin levels.

Striking effects were observed on the administration of ferrous sulphate (Feosol§). Doses smaller than 6 grains daily were ineffective. However, 6 grains or more caused a sharp drop in the plasma ascorbic acid level as will be seen from Fig. 2.

Exactly the same type of response was observed when ferrous sulphate was given to 12 patients receiving bismuth therapy. The greatest drop in plasma ascorbic acid level occurred in patients taking



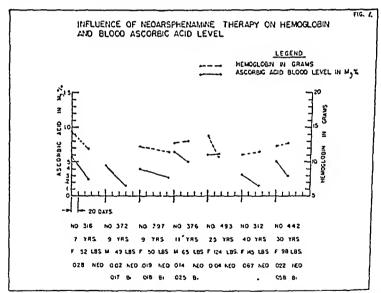
Patients who were at a rest period and did not receive antiluctic treatment were given iron orally in the form of ferrous sulphate ("Feosol" tablets, each containing 3 grains of ferrous sulphate). The hemoglobin rose in every case examined. However, when the dosage of "Feosol" amounted to 2 or more tablets per day there was a sharp drop in the plasma ascorbic acid level in 6 out of the 7 patients.

[§] We are indebted to Smith, Klein and French Laboratories, Philadelphia, for a liberal supply of Feosol Tablets. Each tablet contains 3 grains of ferrous sulphate. The other ingredients are of no significance here.

the fact that in certain hypersensitive cases ascorbic acid gave favorable results after other methods of detoxification such as the administration of glucose, invert sugar, and calcium or sodium thiosulfate had failed.

After a suitable method for determining plasma ascorbic acid had been developed, studies were commenced in 1938 on syphilitic patients showing symptoms of intolerance to arsenicals. A more extended systematic study of this problem was recently made possible in connection with our Nutritional Survey of the syphilitic patients attending the Municipal Social Hygiene Clinic, Chicago.*

It was noted early in the work that patients hypersensitive to neoarsphenamine in whom treatment had to be discontinued because of severe reactions; required exceedingly large oral doses of ascorbic acid; to bring their plasma levels up to optimal values (1.0 mg %



These data demonstrate that neoarsphenamine (in contrast to bismuth) exerts a depressive action on the plasma ascorbic acid level. In 6 out of the 7 cases a distinct decrease in the plasma level occurs. The hemoglobin is but slightly affected.

^{*}We are indebted to Dr. O. C. Wenger, Senior Surgeon, U. S. Public Health Service, and to Dr. Herman N. Bundesen, President of the Chicago Board of Health, for the facilities and opportunity of studying patients attending the Municipal Social Hygiene Clinic. We also wish to acknowledge the cooperative assistance of Dr. G. G. Taylor, Director of the Clinic.

[†] These reactions consisted of nausea, vomiting, fever, dermatitis, and in one case hepatitis.

[†] We are indebted to Merek and Company, Inc., Rahway, N. J., for a generous supply of Cebione (ascorbic acid) used in this investigation.

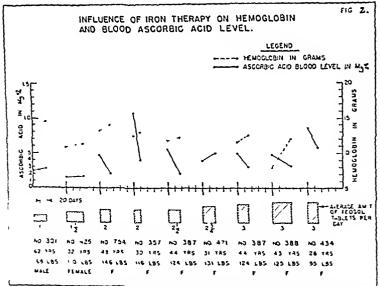
or above). When showing severe symptoms of intolerance, a decline of the plasma level occurred in spite of the oral administration of ascorbic acid during treatment. It was frequently observed that a marked lowering of the plasma level followed the administration of neoarsphenamine in patients showing no intolerance to the drug (Fig. 1).

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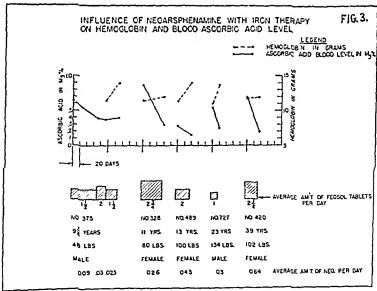


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[§] We are indebted to Smith, Klein and French Laboratories, Philadelphia, for a liberal supply of Feosol Tablets. Each tablet contains 3 grains of ferrous sulphate. The other ingredients are of no significance here.

ferrous sulphate while receiving neoarsphenamine. These data are presented in Fig. 3. It should be emphasized that in spite of this decline in plasma ascorbic acid level the hemoglobin rose in practically every case. This observation is in good accord with the findings of Moore, Bierman and Minnich, who noted a definite decrease in plasma ascorbic acid following a rise in serum iron and hemoglobin after ingestion of large amounts of ferrous or ferric salts.

Our observations suggest 3 types of action of drugs containing heavy metals on plasma ascorbic acid. Bismuth is without influence. Iron causes a marked decrease, which may be of significance in rapid hemoglobin formation. Arsenic in drugs as neoarsphenamine, lowers plasma ascorbic acid, which in some cases may be an attempt on the part of the organism to detoxify the drug. As evidence of detoxification, patients previously hypersensitive to arsenicals have been permitted to resume treatment upon administration of suitable doses of ascorbic acid, when the optimal plasma level was attained. Repeated plasma analyses must be made to determine the amount



These data present the simultaneous action of neoarsphenamine and Feosol on plasma ascorbic acid level and hemoglobin. It will be seen that plasma ascorbic acid values dropped from fairly normal to decidedly low levels. The hemoglobin, on the other hand, rose more or less in every case.

¹² Moore, C. V., Bierman, H., and Minnich, V., Centr. Soc. Clinic. Res., 12th Ann. Meet., 1939.

of ascorbic acid required for maintenance of the optimal plasma level during treatment. In conclusion our data indicate the necessity for a high ascorbic acid intake during certain types of medication with heavy metal compounds, to meet excessive requirements either for physiological demands, or for detoxification of drugs in certain cases before therapeutic levels can be attained.

We wish to acknowledge our indebtedness to H. J. Fagen and J. Meyer for much of the analytical data reported here.

11506 P

Respiratory Metabolism of Pigeons after Adrenalectomy and its Increase by Prolactin.

OSCAR RIDDLE AND GUINEVERE C. SMITH.

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Recent experience in the maintenance of adrenalectomized animals in fair or good condition without use of cortical hormones gives new interest and value to measurement of the basal metabolism of such animals, and the adrenal-pituitary relationship is now the subject of much investigation. The pigeon has been found useful in such studies. It seems to survive complete adrenalectomy readily, though it is best to do the operation in two stages and to inject desoxycorticosterone just before the second operation; thereafter pigeons maintain themselves well without special nutritional or hormonal supplements. Repeated metabolism measurements have been made on 12 such pigeons of various races (both sexes) and on 4 of these birds the ability of prolactin to increase the B.M.R. was demonstrated

Thirty measurements made at 30°C indicate that adrenal removal in pigeons has little effect on heat production; a decrease of 6% was found. Measured at 25°C this decrease was also 6%. Respiratory quotients obtained after a 24-hour fast were the same in operated (0.73) and intact pigeons. In 10 tests made on birds from which a single adrenal was removed no significant effect was observed. The effect of adrenalectomy on the metabolism of the bird is thus found to be less though similar in direction to that previously reported by others for certain manimals. Interpretation of depressions noted in most manimalian tests is usually obscured by the regimen imposed to insure survival or by poor condition of the animals. In pigeons it seems probable that the observed small decrease in B.M.R. was the indirect result of some (presumptive) reduction in bodily activity and of some protection against low temperature. One thyroidectomized pigeon showed a 10% decrease in metabolism following adrenalectomy. Body weights may be well maintained for at least a few months. Measurements were made at intervals varying from 2 days to 4 months after operation.

Four adrenalectomized pigeons with an average B.M.R. of 3.78 calories per kilo-hour were injected with 5 mg (20 units) prolactin daily for 3 days. The heat production of these 4 birds was then +7. +34. +51 and +56. The preparation used (495H2) had been heated to 60°C, at pH 8.0, for 5 hours. This treatment should have been particularly adverse to glycotropic and growth factors if they were present. The prolactin used gave no apparent increase in dove thyroids, and contained little FSH. One of the 4 pigeons used in this test was completely thyroidectomized, and had previously shown no increase in B.M.R. after 5 and 8 daily injections of 4 mg of a prolactin-free preparation of thyrotropic hormone. In this thyroidectomized-adrenalectomized pigeon prolactin increased the B.M.R. by 34%. Earlier studies^{1, 2} have shown that prolactin increases the B.M.R. of hypophysectomized and thyroidectomized pigeons when measured at 30°C. Contrary to an earlier assumption² the present tests indicate that this action of prolactin is not mediated in part by the adrenals.

¹ Riddle, O., Smith, G. C., Bates, R. W., Moran, C. S., and Lahr, E. L., Endocrinol., 1936, 20, 1.

² Riddle, O., Smith, G. C., and Dotti, L. B., Am. J. Physiol., 1938, 123, 171.

11507 P

Cyanosis Produced by Anastomosis of Pulmonary Artery to Left Auricle.

MILTON MENDLOWITZ AND ALAN LESLIE. (Introduced by G. Shwartzman.)

From the Laboratories of the Mount Sinai Hospital, New York City.

Cyanosis, such as occurs in congenital heart disease, is caused by a shunt of unoxygenated blood from the right side of the heart to the left. In order to reproduce this experimentally, it is apparent that a communication must be made at a point where unoxygenated is at a higher pressure than oxygenated blood. The only surgically accessible site for this procedure is the point of contiguity of the main and left pulmonary artery on the one hand, and the pulmonary vein or the left auricular appendage on the other. It was found that because of its relatively small size, the pulmonary vein could not be used.

The procedure therefore employed is as follows: A large dog anesthetized with sodium pentobarbital is placed on its right side, artificial respiration being maintained by intermittent positive pressure insufflation via a trans-oral tracheal cannula. An incision is made in the fourth left intercostal space, the ribs retracted, and the lung packed posteriorly. The pericardium is then incised posterior and parallel to the phrenic nerve, thereby exposing the site for the anastomosis. After isolation of the left pulmonary artery by dissection, an especially designed U-shaped serrefine is placed longitudinally on the main and left pulmonary artery. Blood flow to the right lung is not impaired by this maneuver. A rubber covered short intestinal clamp is then applied to the base of the left auricular appendage. Parallel incisions are now made in the isolated portions of the artery and auricle, which are anastomosed with a vaselinized arterial silk suture on an atraumatic needle. Approximation of intima to endocardium is accomplished by the use of an everting, continuous mattress suture. After the operation the aminal is heparinized, according to the method of Murray, et al.

This procedure has been performed successfully in 3 dogs, in each of which pronounced cyanosis of the tongue and depression of the oxygen content of the arterial blood were observed. Long term observations on these animals, as well as further experiments are now in progress.

¹ Murray, D. W. G., Jaques, L. B., Perrett, T. S., and Best, C. H., Surgery, 1937, 2, 163.

11508

Diuretic Effect of Progesterone.*

HANS SELYE AND LUCY BASSETT.

From the Department of Anatomy, McGill University, Montreal, Canada.

In the course of various experiments in which progesterone had been administered to normal and hypophysectomized rats we observed that this compound greatly increases urine output. We felt that an action of the corpus luteum hormone on diuresis may perhaps throw some light on the cause of the changes in water metabolism observed during normal and pathological pregnancies. Therefore this effect has been subjected to a quantitative analysis in normal and hypophysectomized rats whose urine was collected daily in metabolism cages.

Twenty-four albino females with an average weight of 105 g have been divided into 4 groups of 6. Group I was hypophysectomized and then given daily subcutaneous injections of 10 mg of progesterone in 0.4 cc of peanut oil. Group II was hypophysectomized and given daily subcutaneous injections of 0.4 cc of peanut oil only. Groups III and IV remained intact but received the same injections as Groups I and II respectively. Since hypophysectomy in itself is usually accompanied by a transitory marked diuresis and since the animals need some time to become used to life in individual metabolism cages and daily injections, we did not collect the urine during the first fortnight. After this however, accurate measurements of water intake and output were made on every animal during the 6 subsequent days. Table I shows the urine output during one day. For the sake of brevity the values obtained on other days are omitted since they were essentially the same as those

TABLE I.
24-hour Urine Excretion Under the Influence of Progesterone in Normal and
Hypophysectomized Rats Expressed in cc.

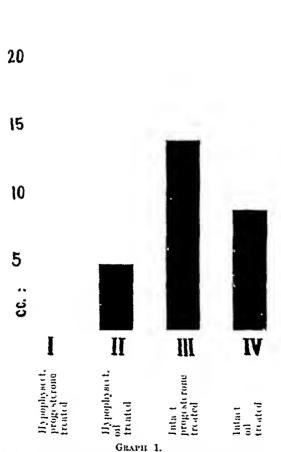
==	Rat No.	1	2	3	4	5	6	Avg
II	Hypophyscetomized progesterone treated Hypophyscetomized oil treated Intact progesterone treated Intact oil treated	11 0 19 6	46 4 16 11	12 2 13 8	21 5 16 9	25 2 15 9	22 10 10 6	23 4 15 8

^{*} The expenses of this investigation were defrayed by a grant received from the Schering Corporation of Bloomfield, N. J., through the courtesy of Dr. G. Straguell. The progesterone was kindly supplied by Dr. E. Schwenk of the same Corporation.

given in the Table. A summary of the average daily output throughout the 6-day observation period is given in Graph I.

We believe that our experiment clearly indicates that progesterone increases the urine output both in intact and in hypophysectomized rats. Although the hypophysectomized controls excreted only about half as much urine as the normals, the progesterone-treated hypophysectomized animals voided much more than any other group in our experiment. The urine output of the individual rats varied somewhat from day to day but it was not uncommon for progesterone treated hypophysectomized animals to excrete during 24

25



Average daily urine output per rat under the influence of progesterone in hypophysictomized and normal animals.

11508

Diuretic Effect of Progesterone.*

HANS SELYE AND LUCY BASSETT.

From the Department of Anatomy, McGill University, Montreal, Canada.

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TABLE I. 24-hour Urine Exerction Under the Influence of Progesterone in Normal and Hypophysectomized Rats Expressed in ce.

	Rat No.	1	2	3	4	5	6	Avg
	Hypophysectomized progesterone treated	11	46	12	21	25	22	23
Π	Hypophysectomized oil treated	0	4	2	5	2	10	4
III	Intact progesterone treated	19	16	13	16	15	10	15
IV	Intact oil treated	6	11	8	9	9	6	8

^{*} The expenses of this investigation were defrayed by a grant received from the Schering Corporation of Bloomfield, N. J., through the courtesy of Dr. G. Stragnell. The progesterone was kindly supplied by Dr. E. Schwenk of the same Corporation.

11509

Effect of Volume Used for Injection in Micro-Assay of Prolactin.

ROBERT W. BATES AND OSCAR RIDDLE

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

The local crop-sac method, or micro-method, of testing for prolactin (intracutaneous injection over the pigeon's crop) introduced by Lyons and Page¹ involves a subjective element and is open to still other sources of error; it has, however, the advantage of extreme sensitivity. Bates and Riddle² reported that subcutaneous injections over the crop-sac area, though they place the prolactin nearer to the responding tissue, are markedly less effective than similar intracutaneous injections. Since the micro-method is currently used in some laboratories for quantitative assay of prolactin, particularly in blood and urine where the concurrent use of 0.1 and 1.0 nil fluid was once advised,¹ it is desirable to learn the sources of error in the use of this method. The present study is concerned with the effect of injecting equal quantities of hormone in unequal volumes of fluid.

In the local crop-sac method the intracutaneously injected prolactin stimulates that area of the crop tissues which lies immediately beneath the site of injection. This fact suggests a direct diffusion from the injection site. If uncomplicated diffusion is involved one would expect the response to be directly proportional to the concentration of prolactin in the solution injected. The crop epithelium is the tissue upon which prolactin acts (by causing cell proliferation) and in passing from the site of injection to this epithelium the hormone must traverse the following very thin structures: lower dermis; a layer of loose connective tissue and fat lying between the crop and the skin; the serosa; and two thin layers of muscle in the crop wall. The crop epithelium is in actual contact with ingested food and the proliferation induced by prolactin occurs in its basal cells.

When 0.05 ml of liquid is injected intracutaneously over the crop it forms a small disk-shaped blister or bleb of fluid, roughly 5 mm in diameter and 2 mm in thickness. Similarly 0.5 ml of liquid forms a blister 16 mm in diameter and 2 mm in thickness. The relative areas are as 1:10, but they have essentially the same thickness. Hence

Lyons, W. R., and Page, E., Peoc. Soc. Exp. Biol. and Med., 1935, 32, 1049.
 Bates, R. W., and Riddle, O., Peoc. Soc. Exp. Biol. and Med., 1936, 34, 847.

hours an amount of urine corresponding to half their body weight. Since the water intake was approximately the same as the output we did not deem it necessary to include the values for water intake in our table.

It seemed of some interest to establish whether increased urine output is merely the result of the increased intake. For this purpose 15 cc of tap water was administered by stomach tube to each rat and then water and food were withdrawn from all animals during 24 hours. Here again the progesterone-treated animals excreted the water more rapidly than the oil-treated controls. This was true both in the case of the intact and the hypophysectomized animals. It is of interest however, that 4 out of 6 oil-treated hypophysectomized controls succumbed from water intoxication after the administration of the relatively large amount of 15 cc while no casualties occurred in any other group. This is in accord with many previous observations made in this laboratory which indicate that progesterone increases the general resistance of hypophysectomized rats.

We wish to call attention to the fact that experiments performed by the senior author in cooperation with Miss C. Dosne indicate that desoxycorticosterone acetate exerts a diuretic effect similar to that of progesterone. These latter observations will be published in detail in the near future.

Summary. Progesterone increases the urine output both in normal and in hypophysectomized rats. Although the untreated hypophysectomized animal excretes less than the normal amount of urine, progesterone stimulates water excretion much more markedly in the absence than in the presence of hypophysis. In hypophysectomized rats, it may lead to the excretion of an amount of urine corresponding to 50% of the experimental animal's total body weight; a diuresis reminiscent of diabetes insipidus. The resistance of hypophysectomized animals to water intoxication is increased by progesterone.

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injected twice in the same spot and killed 48 hours after first injection. The presence of stimulation was estimated from examination of the stretched excised crop by transmitted and reflected light. Several of the birds were also given colchicine 6 to 8 hours before killing and the presence or absence of accelerated cell division was determined histologically. In all tests the gross degree of stimulation was estimated as plus 4, 3, 2 and 1; ½ was used for cases in which stimulation was doubtful. Degree of stimulation is significant (though arbitrarily determined) and the sum of "plus" values for the 4 birds of each group is tabulated. The value assigned to each individual case was probably affected by the larger area of stimulation which results from the larger volume; we tried, however, to consider thickness only.

Crop-sacs stimulated by 0.5 ml containing 0.125 γ in each of 2 injections (total, 0.25 γ) responded as much on the average as crop-sacs stimulated by 0.05 ml containing 0.5 γ (total of 1.0 γ). The ratio of concentrations in these 2 cases is 40 : 1. Thus one obtains the wholly unexpected result that a dilution of 10 times increased the effectiveness of the injected prolactin by a factor of 4.

In a third series of tests (Table II) 4 groups of 3 pigeons each were treated as in the preceding tests except that the total dose was given in a single injection (and killed after 48 hours). Identical results on the relation of volume to response were obtained although 4 to 8 times as much prolactin was required to produce any particular grade of response with a single injection. One γ in 0.5 ml produced as much stimulation as 4 γ in 0.05 ml; 2 γ in 0.5 ml produced as much stimulation as 8 γ in 0.05 ml. Comparison of "once"

TABLE II.

Effect of Injection Volume on Response of Crop-sacs to a Single Local Injection of Prolactin with Autopsy 48 Hours Later.

					Res	ults
Total dose prolactin No. 437	No. of birds	Volume, (ml)	Concentration,	Side of crop used (R or L)	No. positive	Sum of plus values
8.0 ₇	3	.5	16	R	3	+ 8
8.0 ₇		.05	160	L	2½	+ 4½
4.0 ₇	3	,5	8	R	2½	+ 4½
4.0 ₇	3	,05	80	L	1	+ 1
2.0 ₇	3	.5	4	R	21/4	+ 4½
2.0 ₇	3	.05	40	L	1/4	+ ½
1.0 _γ 1.0 _γ	3	.5 .05	2 20	R L	2 0	+ 3

the concentration per unit area may be considered independent of the volume of fluid injected. It is our experience that when stretched for examination the area of crop epithelium stimulated is 5 to 10 mm larger in diameter than the area of the skin displaced by the injected fluid, and that this area is located directly beneath the site of injection. If the mode of transfer of prolactin is by diffusion alone a prolactin solution which produces minimum stimulation when a volume of 0.5 ml is injected should also show minimum stimulation (over a smaller area) when a volume of 0.05 ml of the same solution is injected. The following experiments were designed to test this theory along with a determination of effects of unequal volumes on micro-assay.

Experimental. In tests made with 2 daily injections into the same site 7 groups of 4 birds each were used. Each bird was injected intracutaneously over both crop-sacs with the same amount of prolactin but on one side the volume was 10 times that on the other (0.5 and 0.05 ml); thus the concentration was 10 times greater in the smaller volume of solution. The right crop-sac of all birds of 4 of the 7 groups received the larger volume (Series A, Table 1). The left crop-sac received the larger volume in all birds of 3 other groups (Series B, Table I). The 2 sides of the crop were thus shown to be equally responsive. Each bird was

TABLE I.

Effect of Injection Volume on Response of Crop-saes to Local Intradermal Injections of Prolactin. Both Sides of Crop (R and L) Injected Twice at 24-hour Interval with Autopsy 48 Hours After First Injection.

						Re	sults
Total dose prolactin No. 437	No. of birds	Daily volume, (ml)	Concentration,	Series	Side of crop used (R or L)	No. positive	Sum of plus values
2.0 _γ 2.0 _γ	4 4	.5 .0 <i>5</i>	20	A A	R L	4 4	+12 + 6
1.0 γ 1.0 γ 1.0 γ 1.0 γ	4 4 4	.5 .5 .05 .05	1 1 10 10	A B A B	R L L R	4 4 2 2½	+10 + 6 + 2 + 2½
.5 y .5 y .5 y .5 y	1 1 4 1	.5 .5 .05 .05	0.5 0.5 5.0 5.0	A B A B	R L L R	2½ 3½ 1½ 0	+ 4½ + 3½ + 1½ + 0
.25 ,25 ,25 ,25 ,25 ,25	4 4 4	.5 .5 05 .05	0.25 0.25 2.5 2.5	A B A B	R L L R	3½ 1½ 1½ 0	+ 6 + 1½ + 1½ + 0

injected twice in the same spot and killed 48 hours after first injection. The presence of stimulation was estimated from examination of the stretched excised crop by transmitted and reflected light. Several of the birds were also given colchicine 6 to 8 hours before killing and the presence or absence of accelerated cell division was determined histologically. In all tests the gross degree of stimulation was estimated as plus 4, 3, 2 and 1; ½ was used for cases in which stimulation was doubtful. Degree of stimulation is significant (though arbitrarily determined) and the sum of "plus" values for the 4 birds of each group is tabulated. The value assigned to each individual case was probably affected by the larger area of stimulation which results from the larger volume; we tried, however, to consider thickness only.

Crop-sacs stimulated by 0.5 ml containing 0.125 γ in each of 2 injections (total, 0.25 γ) responded as much on the average as crop-sacs stimulated by 0.05 ml containing 0.5 γ (total of 1.0 γ). The ratio of concentrations in these 2 cases is 40 : 1. Thus one obtains the wholly unexpected result that a dilution of 10 times increased the effectiveness of the injected prolactin by a factor of 4.

In a third series of tests (Table II) 4 groups of 3 pigeons each were treated as in the preceding tests except that the total dose was given in a single injection (and killed after 48 hours). Identical results on the relation of volume to response were obtained although 4 to 8 times as much prolactin was required to produce any particular grade of response with a single injection. One γ in 0.5 ml produced as much stimulation as 4 γ in 0.05 ml; 2 γ in 0.5 ml produced as much stimulation as 8 γ in 0.05 ml. Comparison of "once"

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					Res	ults
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8.0 y	3	.05	160	L	$2\frac{1}{2}$	+ 4%
4.0 _Y	3	.5	8	R	21/2	+ 41/
4.0 Y	3	.05	80	L	1	+ 1
2.04	3	.5	4	R	21/2	+ 41/2
2.0γ	3	.05	40	${f r}$	1/2	十级
1.0 _Y	3	.5	2	${f R}$	2	+ 3
1.0γ	3	.05	20	\mathbf{L}	0	+ 0

injected groups with "twice" injected groups indicates a quadrupling of effectiveness in the latter case.

Discussion. Our observations do not support the assumption that local stimulation of the crop-sac is due to uncomplicated diffusion of prolactin. The volume of fluid injected seems to have an influence or control on the sensitivity of the reaction and this influence is far greater than that which is expected. This may indicate that, in response to the irritation produced by the injection, some substance released in the skin serves to augment the effect of the prolactin. This assumption is supported by the low effectiveness of prolactin injected subcutaneously over crop-sacs, and by other current observations made in this laboratory which show that substances which irritate or insult the skin at the site of injection will cause some cellular proliferation (confirmed histologically) of the crop epithelium. To date none of those irritating substances induce such proliferation when injected systemically.

Calculations of the relative sensitivity of the local and the systemic methods are of interest. Systemic assays of prolactin No. 437, the preparation used in all our tests, showed it to contain 5 Riddle-Bates (or 5 International) units per mg. This is equivalent to about 1.5 to 2.0 systemic minimum stimulating doses (M.S.D.; 50% positive) per mg in a 450 g pigeon (i.e., 500 to 700 $\gamma = 1$ M.S.D.). Using 2 injections, each of 0.5 ml volume, over the crop-sac the M.S.D. of No. 437 was found to be not more than 0.25 y; when a single injection was employed 4 times as much projectin (1γ) was required. These values thus indicate an increase in sensitivity of at least 500 and 2,000 times for single and double injections, respectively. The magnitude of these increases agrees well with Lyons's value of 1,000 for a single injection; but it differs greatly from results of Bergman, Meites and Turner' who report an increase of only 178 although they used the still more sensitive 4-day test (of Lyons). From such 4-day tests Lyons' reported an increased sensitivity of 10,000 times.

The importance of volume of fluid injected is not appreciable in systemic injections by the intramuscular route.

Summary. In 2 types (single and double injection) of 48-hour micro-methods for assaying prolactin, in which the injection volume was 0.05 ml, 4 times as much prolactin was required for minimum stimulation as when the volume was 10 times larger, 0.5 ml. A

³ Lyons, W. R., Proc. Soc. Exp. Biol. and Med., 1937, 35, 645.

Bergman, A. J., Meites, J., and Turner, C. W., Endocrinol., 1940, 26, 716.

⁵ Lyons, W. R., Cold Spring Harbor Symposia on Quant. Biol., 1937, 5, 198.

minimum stimulation dose of prolactin in 0.05 ml thus has its apparent effectiveness increased 4 times by a dilution of 10 times. No simple explanation of this result is apparent. Micro-assays of prolactin by intracutaneous injections over crop-sacs must utilize a constant volume of fluid to be of much quantitative value.

11510

Use of Illuminating Gas to Check Metabolism Apparatus.

IVAN BUNNELL AND FRED R. GRIFFITH, JR.

From the Department of Physiology, University of Buffalo, Buffalo, N. Y.

Combustion of alcohol, ether or acetone is standard procedure to check the operation of metabolism apparatus (Carpenter, et al.¹). We have found it impossible, however, to devise a burner by which the combustion of any of these would proceed evenly for metabolically significant periods at rates comparable to the respiratory metabolism of the rat. On the other hand, small validity would seem to attach to a test several times more severe than planned capacity; failure would be no indication of inability of the apparatus to do what it was designed to do; nor would success be any guarantee that it could perform the more delicate task for which it was made. A micro-balance is not checked with kilogram weights.

As a result, recourse has been had to combustion of gas which can be successfully controlled at almost any desired rate. It was originally intended to use a pure, commercial preparation of one of the lower hydrocarbons in order to eliminate the necessity of control determinations. Preliminary work with ordinary illuminating gas from the city mains was so satisfactory, however, that it has been adhered to; especially since equipment was at hand for the necessary control determinations which involve only slightly additional work.

Since the only difficulty in the application of this principle is accurate measurement of the small volume of gas burned, description of an apparatus which has been found accurate and simple to operate and is easily assembled from odds and ends about any laboratory may be of interest.

This apparatus is shown diagrammatically in Fig. 1.

¹ Carpenter, T. M., Fox, E. L., and Sereque, A. F., J. Biol. Chem., 1929. 82, 335.

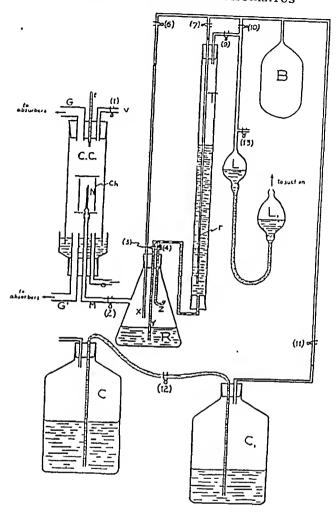


Fig. 1

The gas sample. Since the composition of the city gas supply is not absolutely constant, gas is not burned directly from the supply line. Instead, a sufficient sample for several runs and their attendant control determinations is secured in the 18 liter carboy (C_1) by water displacement into the similar carboy (C). Conversely, as needed, the gas is displaced from (C_1) into the rubber football bladder (B) by siphoning of water from (C) to (C_1) .

The combustion chamber, (C-C) 5.5 cm in diameter and 25 cm long, takes the place of the animal chamber of the metabolism

apparatus (Schwabe and Griffith²). The base of the chamber (S) is the upper part of a wide-mouth bottle and contains mercury for an air-tight seal. Tube (O) connects with the oxygen measuring device of the metabolism apparatus and admits pure oxygen as needed. Tubes (G) and (G_1) connect with the carbon-dioxide absorbers of the metabolism apparatus, by which, also, circulation of air within the chamber is maintained. (t) is a thermometer and (V) a tube which is open during assembly of the chamber to equalize

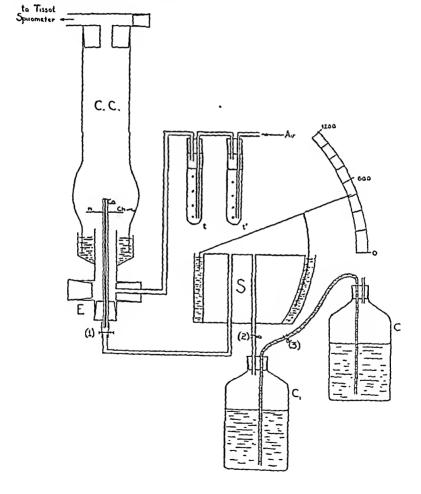


Fig.2.

² Schwabe, E. L., and Griffith, F. R., Jr., J. Nutrition, 1938, 15, 187.

pressure. The illuminating gas is admitted through (M) to whose end, within the chamber, a 26-gauge hypodermic needle (N) which serves as burner, is attached by rubber tubing. The burner (and flame) is protected from air-currents by the chimney (Ch).

Gas measurement is made by the assembly consisting of the oneliter suction flask (R) and the tube (T) which is 2.5 cm in diameter and 100 cm long. It is merely an adaptation of the "microspirometer" of Hanana similar to that used by Schwabe and Griffith to measure the rate of oxygen utilization in the metabolism apparatus. At the beginning of a run (T) is full of water and (R) has been filled with gas to be burned through tube (X) from the bag (B). During a run, due to the fact that the end of (r) in (T) is 15 cm above the opening of (Z) in (R), water flows from (T) to (R) through (Z), and gas enters (T) from (B) through (r) (Mariotte principle), thus establishing a slight, constant, positive pressure on the gas in (R). This gas is forced from (R) through (M) to (N) at the tip of which it is burned. By proper adjustment of the screw-clamp (4) water can be made to flow from (T) to (R) and gas from (R) to (N), at any desired rate. (T) is carefully calibrated so that the amount of water siphoning into (R) and therefore the volume of gas which has been burned, can be accurately known.

Procedure. At the beginning all clamps are closed except (11) and (12). Since there is positive pressure on the gas sample in (C₁) this will fill the football bladder (B). Also with clamps (6) and (2) open and the combustion burner (N) open to the room, (R) is thoroughly flushed out to make sure it contains no trace of foreign gas. Clamps (2) and (6) are then closed, as well as (11) and (12).

To set (R-T) for a run, i.e., to get the water from (R) to (T) a negative pressure is established by the leveling bulbs (L) and (L₁). Open (13) and raise (L₁) until (L) is full; close (13). Lower (L₁) below (L); this establishes a negative pressure in (L) which upon opening clamps (9), (3) and (6) will draw water from (R) to (T). Actually, since (T) is 100 cm long, sufficient negative pressure to effect this transfer is obtained with the help of a water-faucet suction pump attached to the upper, open end of (L₁). When this transfer is complete (9) is closed, the suction is stopped and (3) and (6) are closed.

By operating the unit (T-R) as a closed system as is done here

By operating the unit (T-R) as a closed system as is done here and as explained by Schwabe and Griffith who used a similar modification of Hanan's device for measurement of oxygen admitted

³ Hanan, E. B., Science, 1929, 70, 582.

to the metabolism chamber, the water that is employed is kept in constant contact and equilibrium with the gas that is used, thereby preventing error due to solution of the gas in the water.

At this time all clamps are closed.

To prepare for a run, clamps (7), (4) and (2) are opened in this order. Due to the slight positive pressure between (T) and (R), water will immediately begin to siphon from (T) to (R); gas will enter (T) from (B) at atmospheric pressure and will escape from the burner (N) where it is lit. The chimney (Ch) is put in place, the line to the oxygen supply opened, and with clamp (1) open the combustion chamber is put in place. Before closing (V) slight suction is applied to it to draw enough pure oxygen into the chamber to make sure the flame will burn; clamp (1) is then closed.

Control determination is effected by the set-up shown in Fig. 2. A sample of the same gas is transferred from the carboy (C_1) to the small one-liter, Krogh spirometer (S) and burned at the tip of the 1 mm capillary glass tube (Ca) in the combustion chamber (CC). The flame is protected by the baffle (n) from the current of air (from the compressed air supply) which enters the combustion chamber after being washed through NaOH $(t-t_1)$ and

TABLE I. Results.

		Rate gas burned, cc/min	R.Q.	Ratio CO ₂ /gas	Ratio O ₂ /gas	Rate of O ₂ utilization, cc/min
1/9/40	E* Ct	5.0	.51 .49	.82 .79	1.60 1.60	8.00
1/9/40	C C	10.0	.52 .49	.82 .79	1.58 1.60	16.00
1/22/40	E C	5.5	.51 .52	.84 .87	1.68 1.66	9.25
2/5/40	$_{E}^{\mathrm{C}}$	5.1	.49 .50	.79 .83	1.57 1.68	8.01
2/13/40	E C	5.2	.49 .50	.85 .85	1,72 1.71	9.15
4/22/40	C E	6.5	.52 .50	.84 .86	1.62 1.73	10.53
Average	C E		.51 .50	.83 .83	1.63 1.66	

^{*}Refers to results obtained when a small flame was burned in the Schwabe-Griffith Metabolism Apparatus.

thefers to results obtained when the products of combustion of a larger flame were determined with the Tissot spirometer and gas analysis.

on leaving (at the top of CC) is collected and measured in a 100-liter Tissot spirometer. This air is then analysed in Haldane apparatus.

Comparison of the results obtained in a few of the more recent "experimental" and "control" determinations is shown in Table 1.

11511

Studies on Intercostal Nerve Physiology.

ALFRED J. KAHN. (Introduced by Arno B. Luckhardt.) From the Hull Physiology Laboratory, University of Chicago.

Pathological cases of processes involving the parietal pleura and the diaphragmatic pleura innervated by the lower intercostal nerves, and the attendant referred pain and muscular rigidity in the abdominal region (lower quadrant), have long suggested neural associations between the regions involved. Capps' competent studies upon such clinical cases have yielded valuable information relating the portion of pleural membrane or diaphragm stimulated to site of referred pain.

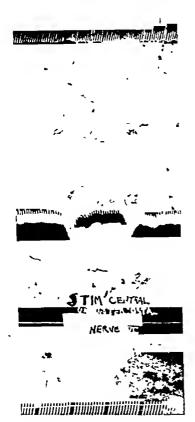
It is a well established fact known to clinicians that: "The abdominal symptoms of thoracic disease are often so misleading that needless or harmful surgery may be carried out. A pleurisy may give referred pain over the distribution of the 6 lower intercostal nerves, with fever may simulate appendicitis."

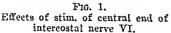
In attempting to establish a physiological basis for such phenomena, we have investigated the intercostal nerves of dogs. The effects of our various manipulations upon blood pressure and respiration were kymographically recorded, the blood pressure obtained from the carotid artery, and respiratory movements recorded by the usual pneumograph-tambour method. In cases where a pneumo-thorax was unavoidable, artificial respiration was administered. Isolation of various intercostal nerves and their branches was facilitated by appropriate rib resection. A tetanizing current was used for nerve stimulation.

Stimulation of the central end of any intercostal nerve results in a transient lowering of blood pressure (approximately 25 mm Hg.)

¹ Capps, J. A., An Experimental Study of Pain, Macmillan, 1932.

² Nelson, Living Medicine, Thomas Nelson and Sons, 1920-1937, 5, 222.







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Fig. 2.

After double vagotomy. Inhibition of respiration by inflation and deflation of lungs. Arrow points to artifact of art. resp.

and an inhibition of respiration for a short interval of time. The interval of respiratory inhibition varied in length from 3 to 5 seconds. Normal respiration is resumed after this interval in spite of continuous stimulation. In some cases a decided decrease in amplitude of respiratory excursions was the only respiratory change observed. It was found that the lower intercostal nerves gave rise to more marked respiratory effects than the upper ones.

In attempting to relate the aforementioned effects to specific branches of the intercostal nerves, it was found that the effects on blood pressure and respiration may be elicited by stimulating the central ends of intercostal branches innervating the diaphragm, parietal pleura, and rectus abdominis muscle. Stimulation of the central end of a branch of the intercostal innervating the diaphragm elicits a spasm of the rectus abdominis muscle. Since this reflex is also elicited by stimulation of the central end of the main trunk of a lower intercostal nerve (6-12), and the reflex is not abolished until all of the lower intercostals are severed, regardless of the order in which they are severed, the efferent fibers of the reflex arc must traverse several of the lower intercostal nerves (trunks X-XII especially). This striking reflex is undoubtedly the cause of the increased tonus of the rectus abdominis muscle observed in many cases of lobar pneumonia and pleurisy.

The effect of the parietal pleural nerve fibers upon respiration suggests a normal regulatory mechanism of respiration caused by the mechanical rubbing of the 2 pleural membranes during respiratory movements. Such a regulatory mechanism was also suggested by the work of Scott, Gault, and Kennedy's who recorded action potentials from the peripheral end of an intercostal nerve during cliest expansion. Pike and Coombs* likewise obtained evidence pointing to a regulatory mechanism of respiration by thoracic and cervical dorsal nerve root section. We sectioned the vagi and phrenic nerves and strongly inflated the lungs with air, whereupon respira-tion ceased for about 20 to 25 seconds. After respiratory movements had started, the lungs were allowed to deflate, and another inhibition of respiration ensued, due to the mechanical stimulation of the parietal pleura by the deflating lungs. The extent of this inhibition with double vagotomy and phrenicotomy was approximately 10-15 seconds. Inflating a balloon in the chest cavity and thus expanding the chest wall internally results in the same respiratory effects. When the phrenic nerves are allowed to remain intact, the intervals of inhibition are greatly reduced (2-5 seconds), and a high cord section (at first thoracic segment) completely abolishes the inhibition. The effect of the phrenic nerves is due to the afferent fibers in them increasing the respiratory rate, as shown by Robb and Deason.5

We believe that the intercostal nerves reinforce the Hering-Breuer reflex in normal respiration. The intercostal nerves may also be a factor in regulating the rate of the respiratory excursions with changes in amplitude, that is, the greater the stimulation of the intercostal fibers, the greater the interval to the next respiratory movement.

³ Scott, Gault and Kennedy, Am. J. Physiol., 1922, 59.

⁴ Pike, F. H., and Coombs, Helen C., Am. J. Physiol., 1922, 59.

⁵ Robb and Deason, Am. J. Physiol., 1911, 28.

Summary and Conclusions. 1. Stimulation of the central end of any intercostal nerve causes a reflex inhibition of respiration and effects a concomitant drop in blood pressure. 2. The lower intercostal nerves (7-12) elicit a greater response than do the upper ones. 3. Stimulation of the intercostal branches to the parietal pleura, diaphragmatic pleura, and rectus abdominis muscle give the respiratory inhibition and lowered blood pressure (approximately 25 mm of Hg.) 4. Stimulation of sensory or intercostal fibers in the diaphragm causes reflex contraction of the abdominal musculature through reflex connection with other lower intercostal nerves. 5. Among other things, these results furnish the physiological mechanisms involved in referred pain and muscular rigidity in the lower abdominal quadrant as a result of involvement of the base of the lungs, in lobar pneumonia for example. 6. Both expiration and inspiration cause the intercostal nerves to be stimulated and thereby effect reflexly respiratory inhibition. a. Inspiration more strongly inhibits respiration than does expiration. b. At the end of inspiration, the intercostal nerves aid the Hering-Breuer reflex. c. After expiration the intercostal nerves constitute a factor that determines lapse of time before the next inspiration. 7. Abnormal respiration and tightening of the abdominal musculature may be indicative of an irritation in the peripheral region of the diaphragmatic pleura.

I hereby wish to acknowledge that Dr. Arno B. Luckhardt suggested the problem and rendered valuable aid in its prosecution.

11512

H Ion Concentration of Various Fluids of the Genital Tract of the Cow.

HENRY A. LARDY, W. D. POUNDEN AND PAUL H. PHILLIPS.

From the Departments of Biochemistry and Veterinary Science, College of Agriculture, University of Wisconsin, Madison.

In a study of certain reproductive phenomena in dairy cattle a few questions were raised which made it necessary to determine the pH of various fluids of the genital tract of the cow. This problem is of considerable scientific and practical interest since it is reported by Warren¹ and others that sex can be controlled by the simple ex-

¹ Warren, Carl, Inimal Sex Control, Orange Judd Co., 1940.

		pH	:
Fluid	No. of cases	Range	Avg
Vaginal douche (Anestrus)	3	6.0-6.7	6.4
Cervical fluid	17	7.6 - 8.9	8,33
Uterine wash*	13	6.6 - 7.15	6.8
Amniotic fluid from calf fetus	ű	7.0-7.4	7.13
Follicular fluid	3	7.52-7.7	7.6

TABLE I. The pH of Various Fluids of the Cow.

pedient of acid or alkaline vaginal douches at the time of breeding. The fluids in our studies were obtained as follows:

Specimens of vaginal and cervical fluid were collected from cows in the University herd by means of a speculum and pipette. The other fluids were obtained from cows immediately after slaughter. The pH determinations were made with the Coleman glass electrode apparatus.

In the cow there is very little vaginal secretion during anestrus. The data obtained upon the pH of the various portions of the genital tract of the cow are summarized in Table I.

During estrus a cord of heavy gelatinous mucus is secreted from the cervix into the vagina. This secretion is distinctly alkaline with an average pH of 8.3 and as it flows into the vagina it changes the reaction in that organ to a slightly alkaline one. For this reason the vagina of the cow in estrus is alkaline. These observations support the findings and hypotheses of McNutt2 et al. This reaction is quite different from the reaction found in the rat, according to Beilly,3 where the vaginal fluid is most acidic during estrus.

From the data given in Table I the sequence of events in the impregnation of the cow would indicate that the sperm are ejaculated into an environment with a slightly alkaline reaction. From there the sperm must pass through the cervical gateway in a medium whose pH normally lies between 8.0 and 9.0. After passing through the cervix the sperm arrives in the uterus which maintains an environment at an average pH of 6.8. This is the pH range which we have found to be optimum for bull sperm storage.4 Thus all sperm on an impregnation journey in the normal cow pass through an alkaline bath before arriving in the uterus where a more optimum pH prevails. The pH of solutions of pure sodium bicarbonate is

^{*5} ec double distilled water was washed through the uterus and the pH of the wash determined.

² McNutt, S. H., Schwarte, L. H., and Eveleth, D. F., Cornell Vet., 1939, 29, 415.

³ Beilly, J. S., Endocrinology, 1939, 25, 275.

⁴ Phillips, P. H., and Lardy, H. A., J. Dairy Sci., 1940, 23, in press.

about 8.8 which lies within the range of alkalinity of the cervix during heat. Furthermore, 10 cc of cervical fluid with a pH of 8.3 requires 3 to 4 cc of N/10 HCl to bring it to pH 6.5. Thus the advocates of pH controlled vaginal douches for the control of sex must go beyond the cervix to make their procedure effective in the female bovine.

Summary. A study of the pH of the genital secretions of the cow has shown the vagina to be slightly acid in anestrus and slightly alkaline in estrus due to the distinctly alkaline character of the cervical discharge at that time. The fluids of the cervix during estrus average pH 8.3 with a range from pH 7.6-8.9. The fluids present in the uterus during estrus have an average pH of 6.8 which is a favorable one for sperm survival.

11513

Lessened Effectiveness of Bacteriostatic Agents vs. Tuberculous Infection in Rabbits with Impaired Functional Efficiency.

ARTHUR LOCKE, ROSE B. LOCKE AND HELEN SCHLESINGER. From the Western Pennsylvania Hospital Institute of Pathology, Pittsburgh.

This is a report of a study of the effect of administered sulfanilamide (SA) and p-caproylaminobenzenesulfonhydroxamide (CH)* on the rate of increase in size of the local lesion produced by subcutaneous injection of a suspension of avian tubercle bacilli, in the rabbit, and on the bearing—on that effect—of the functional status of the rabbit as indicated by its reaction to chilling.^{1, 2}

Table I indicates the comparative intensity of the growth-restraining effect exerted by SA and CH in cultures of the avian tubercle bacillus in broth. The strain used was M. avium No. 30, from the Phipps Institute. It formed smooth, moist, cream-colored colonies on Lowenstein's medium. In broth, it grew in sedimenting, flocculent masses which formed an even suspension on agitation. The culture medium was glycerine veal heart infusion broth of pH 6.8, distributed in 25 cc amounts in 50 cc Erlenmeyer flasks. The

^{*} Supplied by Dr. Maurice Moore, Sharp and Dohme, Philadelphia.

¹ Locke, A., J. Infect. Dis., 1937, 60, 106; J. Immunol., 1939, 36, 159; Locke, A., and Main, E. R., Ibid., 173.

Locke, A., Locke, R. B., Bragdon, R. J., and Mellon, R. R., Science, 1937, 86,
 Locke, A., Main, E. R., and Mellon, R. R., J. Immunol., 1939, 36, 183.

pН Fluid No. of cases Range Avg Vaginal douche (Anestrus) 3 6.0-6.7 6.4 17 Cervical fluid 7.6 - 8.98.33 Uterine wash* 13 6.6-7.15 6.8 Amniotic fluid from calf fetus 7.12 6 7.0-7.4 3 7.52 - 7.7Follicular fluid 7.6

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TABLE II. Comparative Size of the Local Lesion Developed Following Subcutaneous Injection of a Suspension of the Avian Tubercle Bacillus into Treated and Untreated Rabbits Making Optimal and Sub-optimal Reaction to Chilling.

			Per diem wt loss		Approx, area of lesion in cm ² on the indicated day of infection				
Warming time ¹	Rabbit No.	Drug given	in g during treatment	9	20	40	60		
	T	reatment	begun immed	iately af	ter infecti	on.			
30-40	3	none	•	5	5	7			
	1	none		8G	8G	6GD			
	671	CH	5	1	1*	(9)	(6D)		
	679	\mathbf{CH}	0	1	2*	(6)	•		
	2	SA	23	4*	(13)	(13)			
	675	SA	63	4M	()	• •			
>40	681	none		12	12	10D			
	677	none		9G	18G	20GD			
	667	CH	14	11*	(14)	(20GD)			
	664	SA	13	7*	(9)	(12)			
	T	reatment.	begun 20 to 4	0 days a	fter infecti				
30-40	656	none			8	2D	3D		
	639	CH	4		81	10			
	649	CH	õ		41	4	0‡		
	655	CH	0			6t	6D		

^{*}Day of discontinuance of treatment. The subsequent, bracketed values indicate extent of change in lesion size following cessation of treatment.

Day treatment was begun.

invasion and rabbits with warming times appreciably longer than 40, a minimum capacity for survival. Transition from a warming time of 40 or longer, to 33, could be induced through enforced rest, circulatory support and vitamin administration; and converse impairment from 33 to more than 40 through exhaustion, shock and starvation. The ability of SA to check pneumococcus invasion in rabbits with warming times less than 40 was observed to be more than double that observed in rabbits with warming times exceeding 40.2

The warming times of the upper group of rabbits in Table II lay between 30 and 40. Those of the lower group lay beyond 40. New Zealand White males were used, from 3 to 4 kg in weight. They were injected subcutaneously, within a shaved area over the abdomen, with 0.002 to 0.004 cc of sedimented, growing avian tubercle bacilli suspended in 0.5 to 0.75 cc of broth, one or more days after the last preceding estimation of warming time and from 0 to 2 hours before beginning drug feedings. The SA was given in capsule in a dosage of 0.3 g 8 x daily and the CH in a dosage

Excision of the area revealed persisting tubercle bacilli. The notation M means dying. The terminal blood SA level, in this rabbit, 18 hr after the last preceding SA feeding, was 28 mg%. G denotes lymph-node invasion and D, drainage.

TABLE I.

Comparative Effects of Sulfanilamide (SA) and p-caproylaminobenzenesulfonhydroxamide (CH) on the Growth of the Avian Tubercle Bacillus in Broth Culture.

Series	Total No. cultures in group	Drug added	Cone. nillimolar	Avg vol of sediment from 10 ce of culture at 20 days, cc × 1000	Estimated avg % inhibition
2, 3, 5b	15	none		21	
, ,, ,	ĩ	CH	.26	$\begin{Bmatrix} 1 \\ < 1 \end{Bmatrix}$	97
(small inocula)	14	CII	.52	<1 }	
	G	SA	.26	2)	93
	9	SA	.52	<1 }	
1, 4, 5a	18	none		24	
	7	CH	.26	87	60
(heavier inocula)	8	CH	.52	11 {	•••
•	8 7	SA	.26	$2\tilde{0}$ $\{$	20
	11	SA	.52	19 }	_•

drugs were added in 0.1 and 0.2 cc volumes of 60% ethyl alcohol solution. Equivalent additions of 60% alcohol free of dissolved drug produced no recognized growth-checking effect. Ten cc aliquots of the cultures were centrifuged after incubation at 38°C for 20 days. The collected sediments were found to be pure.

The series in the lower group in Table I were inoculated from 3 to 5 times more heavily than those in the upper group. The average percent inhibition exerted by the CH in the upper group was 97; that exerted by the SA, 93. The equivalent averages for the group receiving the larger inocula were 60 and 20. The CH would appear to have been, in this *in vitro* comparison, more effective than the SA but less effective against a relatively large inoculum than against a small one. CH is, mole for mole, also more effective than SA in checking growth of the type I pneumococcus in broth culture but is less effective than SA in cultures containing blood.³ It has approximately the same effectiveness as SA in pneumococcusinfected mice.⁴

The usefulness of the reaction to chilling as an index of functional status, in the rabbit, was established in an investigation of factors determining non-specific capacity for resistance to pneumococcal invasion.¹ Rabbits with "warming times" of 30 to 33 were found to have a maximum non-specific capacity for surviving such

³ Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 593.

⁴ Cooper, F. B., Gross, P., and Lewis, M., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 491.

11514

Sulfapyridine and Serum in Experimental Type III Lobar Pneumonia.

J. L. WRIGHT AND F. D. GUNN.

From the Department of Pathology, Northwestern University Medical School.

Previous experiments¹ have demonstrated that sulfapyridine and sulfanilamide were about equally effective in reducing mortality in Type III lobar pneumonia of rats. Up to the time of publication of those results, the samples of specific antiserum that we had tested had shown no decisive effects in protecting against Type III infections in rats. Within the last year accurately standardized, highly concentrated Type III serums* have been available and in our experiments, when used in large doses, they have exhibited a protective value approaching that of type specific serum in Type I pneumococcic pneumonia.²

In these experiments serum and sulfapyridine were used together and separately in doses which had been found to afford the greatest degree of protection. The optimal quantity of serum was found to be between 1,000 and 2,000 units per rat when the total was distributed over a period of 7 days and that for sulfapyridine between 850 and 1250 mg when given in divided doses over the same period.

One hundred and sixty young adult rats weighing between 140 and 280 g were infected in groups of 40 by the intrabronchial method previously described by Nungester and Jourdonais.³ In each group there were 10 untreated controls, 10 treated with serum by intraperitoneal injection, 10 treated with sulfapyridine, administered by stomach tube, and 10 treated with both serum and sulfapyridine.

The strain of Type III pneumococcus was that used in previous experiments.¹ The dose used in the first 2 groups of 40 rats was 0.1 cc of a 16-hour bouillon culture, diluted to 10⁻⁵. This produced in the untreated controls a mortality of 63%. In the third and fourth groups the dose was increased 10 times (10⁻⁴ dilution) and the initial mortality was 100%.

Treatment was begun in all cases about 4 hours after injection.

^{*} Donated by Lederle Laboratories, Inc., New York, N. Y.

[†] Donated by Merck and Company, Rahway, N. J.

¹ Kepl, M., and Gunn, F. D., Proc. Soc. Exp. Biol. and Med., 1939, 41, 457.

² Kepl, M., and Gunn, F. D., Proc. Soc. Exp. Biol. and Med., 1939, 40, 529.

³ Nungester, W. J., and Jourdonais, L. F., J. Bact., 1935, 29, 34.

of 0.45~g~5~x daily or 0.23~g~8~x daily. Preliminary trial had indicated the ineffectiveness of smaller, less frequent dosage. The drugs could not be given, successfully, in admixture with food.

The infection was not rapidly fatal. The indicated death of rabbit 675 was due to sulfanilamide poisoning, not infection. The SA proved to be so toxic in effective dosage as to prohibit sustained use. Loss of appetite was induced, together with rapid decrease in weight. The comparative non-toxicity of the CH was the determining point in its choice for this study. CH produced no impairment in appetite, significant loss of weight or other directly recognizable injury during sustained, massive feedings over periods of 20 to 40 days. Adjuvant feedings were given, during protracted CH administration, of sodium bicarbonate, ascorbic acid and liver extract.

The lesions produced at site of injection in the control rabbits with warming times of 30 to 40 were, roughly, one half the size of those developed in the control rabbits with warming times longer than 40. A comparable relationship was apparent in the treated rabbits following cessation of treatment. During the period of treatment, the lesions in the 30-40 minute rabbits given CH from the outset of infection were approximately one-sixth the size of those observed in the controls but quickly grew out to normal size following discontinuance. The corresponding lesions in the 30-40 minute rabbits given SA were about two-thirds the size of those observed in the controls. No significant effect of CH or SA was indicated in the rabbits with warming times exceeding 40, either on a basis of lesion size at time of drug discontinuance or on a basis of extent of further development following withdrawal. CH administration begun 20-40 days after infection was without significant checking effect. The infection was in no case eradicated.

Summary. Sulfanilamide and p-caproylaminobenzenesulfonhydroxamide, producing 20 and 60% inhibition of growth, respectively, in broth cultures of the avian tubercle bacillus, exert a comparable degree of restriction on the rate of increase in size of the local lesion produced by subcutaneous injection of this organism, in rabbits with warming times less than 40, but not in rabbits with warming times appreciably longer than 40.

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TABLE I.
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•	Total No. of rats	No. survivors	No. fatalities	% mortality	Therapy	Dilution of organisms
	19*	7	12	63	None	1:100,000
	20	13	7	35	Serum	1:100,000
	19*	14	5	26	Sulfapyr.	1:100,000
	19*	14	5	26	Serum and Sulfapyr.	1:100,000
	20	0	20	100	None	1:10,000
	20	4	16	80	Serum	1:10,000
	18*	11	7	39	Sulfapyr.	1:10,000
	16*	10	6	37	Serum and Sulfapyr.	1:10,000

^{*}In each group 20 animals were originally injected. However, those killed by trauma incurred by the injection of the drug, or those which did not show the ink spot tracer in the lung on post-mortem examination, were deleted from the experiment.

The initial dose of sulfapyridine was 250 mg, emulsified in 2 cc of 1.5% mucin. This was followed by a twice daily dose of 125 mg for 2 days and then 125 mg daily for 4 more days. In the first 2 experiments the initial dose of serum was 250 units in 1 or 2 cc of saline and the maintenance dose was 125 units given twice daily on the second and third days and once a day for the next 4 days. In the third and fourth experiments the method differed only in that the initial dose was 500 units instead of 125 units.

In the table, the first and second groups and the third and fourth groups are combined. Serum therapy alone resulted in a reduction of mortality from 63% to 35% in the 80 rats which were infected with the smaller dose of pneumococci. Under the same conditions, animals receiving sulfapyridine alone showed a mortality of 26%. The same figure is shown for the group receiving both serum and sulfapyridine.

In the 80 rats which were infected with the larger dose (10⁻⁴ dilution) serum therapy reduced the mortality from 100% to 80%; sulfapyridine therapy reduced it to 39%. A combination of sulfapyridine and serum showed no significant improvement over sulfapyridine therapy alone, giving a mortality of 37%. Under the second group of conditions, where the most important factor which has been changed is the dose of pneumococci (increased from a dilution of 10⁻³ to 10⁻⁴) sulfapyridine appears to be about 3 times as effective as serum (survival rate of about 60% for sulfapyridine and about 20% for serum). In unpublished experiments we have found that a further increase of the dose of serum, especially in the first 2 days, does not further reduce the mortality when the infecting dose is large.

Summary. Under the conditions of our experiments the protective value of highly concentrated type specific serum in optimal doses and that of sulfapyridine in optimal doses are approximately equal when the infecting dose of Type III pneumococci is relatively small, resulting in a mortality of 63% in untreated animals. When the infecting dose is sufficiently large to produce an initial mortality of 100%, the mortality after sulfapyridine therapy is significantly less than after serum therapy. Combining serum and sulfapyridine, each in optimal dose, does not reduce mortality below that of sulfapyridine therapy alone in Type III pneumococcic pneumonia, differing in this respect from results previously obtained from similar experiments with Type I pneumococcic pneumonia.

11515

Effect of Sulfanilamide, Sulfapyridine. and Sulfathiazole on Staphylococcus Toxins.*

MILWARD BAYLISS. (Introduced by Martin Frobisher, Jr.)

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Conflicting reports have appeared in the literature on the neutralization of staphylococcus toxins by sulfanilamide and allied compounds. Levaditi and Vaisman¹ were unable to demonstrate any effect of prontosil, neoprontosil, and other azo-sulfonamide derivatives against staphylococcal hemolysin, although they claimed these compounds neutralized the effect of streptococcal leucocidin and hemolysin. Later Levaditi, Vaisman, and Reinie² reported that none of the compounds tested was effective against staphylococcus lethal toxin. Osgood and Powell³ found that sulfanilamide in concentrations of 1:1000 or less did not inactivate significant amounts of staphylococcal hemolysin. Recently Carpenter and his co-

^{*} The author wishes to acknowledge his appreciation of the interest, and the valuable suggestions of Dr. E. K. Marshall, Jr., and Dr. Perrin Long.

¹ Levaditi, C., and Vaisman, A., Compt. Rend. Soc. de Biol., 1935, 120, 1077.

² Levaditi, C., Vaisman, A., and Reinie, L., Compt. Rend. Soc. de Biol., 1937, 126, 1937.

³ Osgood, E. E., and Powell, H. M., Proc. Soc. ENP. BIOL. AND MED., 1938, 30, 37.

workers⁺⁷ have reported an antitoxic effect of sulfanilamide and its derivatives on toxins formed by the gonococcus, pneumococcus, staphylococcus, Streptococcus hemolyticus, Clostridium botulinum, Clostridium tetani, Clostridium septicum (vibrion septique), and Clostridium perfringens. Although in his in vivo experiments he reports in one paper⁵ neutralization, and in another paper,⁷ failure to neutralize staphylococcus lethal toxin, he reports statistically valid in vitro experiments demonstrating consistently the neutralization of staphylococcus lethal toxin.

In the present study, toxins from 4 strains of hemolytic staphylococci were used, as well as one batch of toxin labelled "Lot O" prepared by Carpenter. The effect of sulfanilamide (para amino benzene sulfonamide), sulfapyridine (2-sulfanilyl amino pyridine), and sulfathiazol (2-sulfanilyl aminothiazole) on these toxin preparations was investigated. The toxins were prepared according to the method of Dolman and Wilson.8 A 2-day culture of the organism following growth on semi-solid agar was passed through filter paper, and finally through a Seitz filter to remove the bacteria. M.L.D. of each lot of lethal toxin was determined for adult mice weighing 22 to 26 g. In this study two strains of albino mice and one strain of black mice were used, but only one strain of mice was used in each experiment. Each group, including the control group, contained the same proportion of males and females with comparable weights. The mice were observed for 7 days, although very few deaths occurred after 48 hours. The sulfanilamide or its derivative (in 0.85% sodium chloride solution) was thoroughly mixed with the toxin and 1 cc of the mixture injected intraperitoneally into each mouse from 5 to 45 minutes following mixing. Control mice received 1 cc of the saline solution containing the same amount of toxin.

During initial experiments in which a dose of toxin was administered sufficient to kill 100% of the control mice, it was always found that 100% of the mice injected with toxin-sulfanilamide mixtures also died. Subsequent experiments were therefore per-

⁴ Carpenter, C. M., Barbour, G. M., and Hawley, P. L., J. Pediatrics, 1939, 14, 116.

⁵ Carpenter, C. M., and Barbour, G. M., PROC. Soc. Exp. BIOL. AND MED., 1939,

⁶ Carpenter, C. M., and Barbour, G. M., PROC. Soc. EXP. BIOL. AND MED., 1939,

⁷ Carpenter, C. M., Proc. Third International Congress for Microbiol., 1939,

⁸ Dolman, C. E., and Wilson, R. J., J. Immunology, 1938, 35, 13.

TABLE I.

Effect of Sulfanilamide and Allied Compounds on Staphylococcus Lethal Toxin.

	M	ice injected		
	Total	No. survived	% survival	
Control	150	79	56	
1:100 Sulfanilamide	100	36	36	
1:200	100	39	39	
1:1000 "	120	55	45	
1:1000 Sulfapyridine	120	58	48	
1:1000 Sulfathiazole	120	61	51	

formed in which approximately half of the control mice survived. Table I summarizes the results of the latter experiments. It is evident that rather than neutralizing the lethal action of the toxin, if anything the drugs slightly enhanced the toxicity to mice. It is difficult to reconcile these results with those of Carpenter. It was therefore thought desirable to determine whether these compounds might influence the other toxic activities of staphylococcus toxin.

The neutralization of dermo-necrotic toxin was tested by injecting 4 albino guinea pigs and 4 albino rabbits with staphylococcus toxin plus various dilutions of sulfanilamide and its derivatives. Each animal was injected intradermally with .2 cc of each mixture. The area of necrosis was measured on the fifth day. The average of these measurements is listed in Table II.

It is obvious that there was no neutralization of the dermonecrotic factors present in the toxin.

The effect on the hemolysins was studied by several methods. Using 2 hemolytic units of toxin with the addition of various concentrations of the sulfonamides to a 1% rabbit erythrocyte suspension, it was found that high concentrations of the chemicals reduced the hemolysis partially, whereas concentrations normally attained in the blood during treatment of infections had no effect. This

TABLE II.
Effect of Sulfonamides on Dermo-necrotizing Toxin.

	Avg of areas of necrosis, cm ²
Toxin + saline solution (control)	2.2
" + 1:10,000 sulfanilamide	2.0
" + 1: 1,000 "	2.2
" + 1: 100 "	2.3
'' + 1:10,000 sulfapyridine	2.2
'' + 1: 1,000 ''	2.1
'' + 1:10,000 sulfathiazole	2.2
'' + 1: 1,000 ''	2.0

workers⁴⁻⁷ have reported an antitoxic effect of sulfanilanide and its derivatives on toxins formed by the gonococcus, pneumococcus, staphylococcus, Streptococcus hemolyticus, Clostridium botulinum, Clostridium tetani, Clostridium septicum (vibrion septique), and Clostridium perfringens. Although in his in vivo experiments he reports in one paper⁵ neutralization, and in another paper,⁷ failure to neutralize staphylococcus lethal toxin, he reports statistically valid in vitro experiments demonstrating consistently the neutralization of staphylococcus lethal toxin.

In the present study, toxins from 4 strains of hemolytic staphylococci were used, as well as one batch of toxin labelled "Lot O" prepared by Carpenter. The effect of sulfanilamide (para amino benzene sulfonamide), sulfapyridine (2-sulfanilyl amino pyridine), and sulfathiazol (2-sulfanilyl aminothiazole) on these toxin preparations was investigated. The toxins were prepared according to the method of Dolman and Wilson.8 A 2-day culture of the organism following growth on semi-solid agar was passed through filter paper, and finally through a Seitz filter to remove the bacteria. M.L.D. of each lot of lethal toxin was determined for adult mice weighing 22 to 26 g. In this study two strains of albino mice and one strain of black mice were used, but only one strain of mice was used in each experiment. Each group, including the control group, contained the same proportion of males and females with comparable weights. The mice were observed for 7 days, although very few deaths occurred after 48 hours. The sulfanilamide or its derivative (in 0.85% sodium chloride solution) was thoroughly mixed with the toxin and 1 cc of the mixture injected intraperitoneally into each mouse from 5 to 45 minutes following mixing. Control mice received 1 cc of the saline solution containing the same amount of toxin.

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⁴ Carpenter, C. M., Barbour, G. M., and Hawley, P. L., J. Pediatrics, 1939,

⁵ Carpenter, C. M., and Barbour, G. M., PROC. Soc. Exp. Biol. And Med., 1939,

⁶ Carpenter, C. M., and Barbour, G. M., PROC. Soc. EXP. BIOL. AND MED., 1939,

⁷ Carpenter, C. M., Proc. Third International Congress for Microbiol., 1939,

⁸ Dolman, C. E., and Wilson, R. J., J. Immunology, 1938, 35, 13.

might be one of the main activities of the sulfonamide compounds, and that more potent antitoxic compounds might prove even more useful in the treatment of bacterial infections. These experiments, however, lead one to believe that the only manner in which the toxicity of staphylococci is affected is by an inhibition of growth of the organism with a consequent decreased production of toxin.

Summary. Toxic manifestations of staphylococci are not inactivated in vitro by sulfanilamide, sulfapyridine, or sulfathiazole. The lethal toxin, dermo-necrotic toxin, coagulase, and enterotoxin are not neutralized by solutions of the sulfonamides tested at 37° C. α - and β -hemolysins are slightly diminished in activity at concentrations approaching the saturation point of the sulfonamides, but are unaffected at concentrations of less than .01%. These compounds appeared to decrease hemolysin production by decreasing the growth rate of the organism.

11516 P

Response of Plasma Volume to Diuretics.

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Previous work¹ has led us to conclude that mercurial diuretics act by diminishing tubular reabsorption, while administration of aminophyllin produces an increase in the volume of the glomerular filtrate. Earlier work² suggested a high circulating blood volume in congestive heart failure, but there was no complete agreement as to the change following diuresis. Recent determinations³ by a more satisfactory method⁴ demonstrate a marked elevation of blood and plasma volume in patients with failure, and a decrease towards normal with the development of circulatory compensation. A similar decrease has been noted after the use of mercurial diuretics.⁵

We have followed over 12 to 24 hours the changes in plasma volume, determined by the method of Gibson and Evelyn,4 after the

¹ Herrmann, G., and Decherd, G., J. Lab. and Clin. Med., 1937, 22, 767.

² Goldhammer, S., Leiner, G., and Scherf, D., Klin. Woch., 1935, 14, 1109.

³ Gibson, J. G., and Evans, W. A., J. Clin. Invest., 1937, 16, 851.

⁴ Gibson, J. G., and Evelyn, K., J. Clin. Invest., 1938, 17, 153.

⁵ Harris, Alfred W., personal communication.

	` TABLE III.
	Effect of Sulfonamides on Alpha-Hemolysin.
Rabbit	crythrocytes 1% + 2 hemolytic units staphylococcus hemolysin incubated
	1 hour 37°C.

Concentration, %	.1	.05	.025	.0125	.006*	.0002*	Control
Sulfanilamide Sulfapyridine Sulfathiazole	2+	3+	3+ 2+ 2+	4+ 3+ 3+	4+ 4+ 4+	4+ 4+ 4+	4+ 4+ 4+

^{*}Concentrations of .003, .0015, .0008, and .0004 gave same results as .006 and .0002.

confirms the report of Osgood and Powell,3 and of Gross, Cooper, and Lewis.9 Similar experiments, using sheep erythrocytes with incubation at 37°C for one hour followed by 12 hours at 7°C, gave essentially the same result.

Blood agar plates were prepared containing various concentrations of the 3 chemicals. The results in Table IV demonstrate that decreased production of hemolysin by staphylococci is due mainly to a decreased growth rate.

In a series of experiments to determine the effect of these compounds upon staphylococcus enterotoxin, no demonstrable neutralizing activity was found. Kittens injected intraperitoneally with toxin-sulfanilamide mixtures vomited in the same length of time, and with the same degree of severity as kittens injected with toxin alone.

Using dilutions of 24 hour broth cultures of various strains of staphylococci mixed with various concentrations of the 3 compounds, no significant alteration of coagulase other than might be attributed to reduced growth rate of the organism was observed.

Although numerous statements have been made concerning the mechanism by which sulfanilamide acts, there is very little proof in support of any of these, other than those concerning its bacteriostatic action. The experiments described in this report were performed in the hope that the neutralization of the toxic products

TABLE IV. Effect of Sulfonamides on Hemolysin Production in Blood Agar Plates.

	Control	Sulf	anila 0.1	mide	Sulfapy .01	ridine	Sulfat	hiazole O 1
Concentration % Diameter hemolysis (mm) Diameter colony (mm)	4.5 1.6	3.3		0.3*		2.2*	2.8*	1.2* 0.7

^{*}Indicates partial hemolysis.

⁹ Gross, P., Cooper, F. B., and Lewis, M., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 275.

of the desired myocardial effect. As the rate of urinary flow accelerates, this increase disappears and is followed by a reduction, the amount of which is determined by the rate of diuresis.

The fluid eliminated during a rapid salyrgan diuresis seems to come largely from the plasma during the first 8 to 12 hours. After this period, as the rate of diuresis drops, the plasma volume is partially restored. In one of our cases there was a delayed diuresis, in one no diuretic response to the drug, and the plasma volume was slightly increased in each, suggesting that in addition to the usually dominant renal action, the mercurial also exerted an accessory effect on tissue fluid mobilization.⁵

The administration of 0.48 g of aminophyllin intravenously results in relatively large rise in the plasma volume, amounting in various individuals to from 400 to 1200 cc. The first rise coincides with the time of maximal diuresis; the plasma volume drops slightly during the period of rapid urine flow, but the rise continues as the degree of diuresis abates, and persists for approximately 6 hours. After this time the volume drops sharply, presumably due to a return of fluid into the tissues, for the rate of urine production has by then dropped back nearly to the control level. In one experiment the usual rise in plasma volume was preceded 20 minutes after injection by a slight drop. The significance of this finding must be further explored. The striking increase in plasma volume after aminophyllin injection cannot be adequately explained by the available data. The possible mechanisms for tissue fluid mobilization, as well as the concomitant shifts in total circulating plasma protein⁶ are being further investigated.

⁶ Calvin, D. B., Proc. Am. Physiol. Soc., in press.

TABLE I.
Plasma Volume After Injection of Diuretics.

Hr after drug inj.	Plasma vol, cc	Urine flow, cc/min	Plasma vol, cc		Plasma vol, cc	Urine flow, cc/min
			Salyrgan.			
	L	Ħ	E	T	7	7
_						
0	4634	0.86	5260	0.47	5140	0.53
<u> 1/2</u>	4486	1,17	5520	.70	5110	.85
1	4386	8.00	5000	.79	4620	1.78
1 2 3 5 7 9	4060	11.33	4894	.48	4620	4.58
3			4394	.56	2000	9.50
ე #			4916 5088	.99	3280	3.78
0			4826	1.29 1.31		
11			4820 4045	1.33		
11			Aminophyllin,	1.55		
	Ą			R	A	3
0	3440	1.00	6240	1.05	6120	1.17
1/2		_,,,,	V-1-	2.0-	6488	3.09
1 ~~	3713	1.05	6840	2.87	6383	5.14
1 2 3 5 7	4170	1.70	6720	3.35	6500	5.02
3	3976	1.92	6660	3.14	6697	4.77
5	3537	1.58	6220	2.04	7202	1.80
7	3732		5220	1.70	6278	1.55
9 11	3649				4913	1.08
11	3683	.16	5580	1.20	4072	.60
			Digoxin.	_		
	A	•	H	В	SC	;
0	5070	0.37	3890	0.10	4710	.83
1	5190	.56	4130	4.52	4310	3.84
2	5150	.85	3640	2.24	4237	3.67
3	4970	.91	3682	5.20	4273	1.80
5	5170	.90	3800	2.26	4164	6.08
0 1 2 3 5 7	5140	1.33	3790	3.43	3982	4.50
9	4830	1.70	3060	3.81	3947	8.20
11	4446	2,29	2505	3.28	3645	10.00
24	3820	2.25				

injection intravenously of one of the 3 types of diuretic drugs, salyrgan, aminophyllin, and digoxin. The urinary output has also been carefully followed, and the plasma volume correlated with the degree of diuresis. Data from typical experiments are recorded in Table I. It is to be emphasized that under the conditions of our experiments the plasma volume is influenced by fluid loss through the kidneys and fluid mobilization from the tissues. These factors exert an opposite effect, and their relative magnitude determines the blood volume.

When digoxin is injected in a dose of 2 mg there is first noted a slight increase in the plasma volume. This has never been great, and the time of its appearance seems to depend on the attainment

TABLE I.

	17100		
Groups studied	No. of mice inoculated	No. of mice that died	% of mice that died
	Experin	nent I.	
Control	-		
1	30	20	66
2 3	30	29	96.6
3	30	30	100
			
Totals	90	79	87.7
Experimental			
1	30	25	83.3
2 3	30	30	100
3	30	30	100
Totals	90	85	94.3
	Experim	ent II.	
1	30	4 7	13.3
2	30	7	23.3
3	30	27	90
4 5	30	27	90
5	30	0	0

In the above experiment, Neoprontosil did not protect mice from Cl. zwelchii infection.

Experiment II—A 24-hour glucose broth culture was centrifuged at high speed. The supernatant was set aside and the sediment was repeatedly washed with saline. After repeated centrifugations and washings, enough saline was added to the cells to restore the original volume of the culture, and 0.1 cc of this suspension was used for the inoculations.

One hundred fifty mice were separated into 5 groups of 30 mice each. Group 1 was inoculated intramuscularly with 0.1 cc of washed cells of *Cl. welchii*. Group 2 was inoculated intramuscularly with 0.1 cc washed cells and 0.1 cc filtrate from the same culture. Group 3 was inoculated with 0.1 cc washed cells and 0.1 cc filtrate, plus 1 cc Neoprontosil, Group 4 with 0.1 cc washed cells plus 1 cc Neoprontosil, and Group 5 with 0.1 cc filtrate plus 1 cc Neoprontosil.

Four mice (13.3%) died in Group 1, 7 (23.3%) died in Group 2, 27 (90%) in Group 3, 27 (90%) in Group 4, and none in Group 5. (Table, Exp. II.) In this case, the injection of Neoprontosil with washed cells of *Cl. welchii* led to the development of the corresponding infection, while the washed cells alone showed little tendency to develop in the tissues after inoculation.

In repeating the above experiment, the dose of Neoprontosil was lowered, using 0.75 cc, 0.50 cc and 0.25 cc, and in every case, when the dose of Neoprontosil was given with washed cells of Cl. welchii,

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Effect of Azosulfamide (Neoprontosil) on Experimental Welchii Infection in Mice.

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Bliss and Long¹ report that suifanilamide has a curative effect in mice infected with *Clostridium welchii*. Carpenter and Barbour² report that oral administration of Neoprontosil prevented death in mice given the toxin of *Clostridium welchii*. We decided to study the effect of Neoprontosil (Winthrop Chemical Co.)* on experimental welchii infection in mice.

The strain of Cl. welchii used in the following experiments was isolated from a human case of gas gangrene and is similar, in its biological characteristics, to the classical Cl. welchii described in textbooks. The culture was passed through Swiss mice before culturing in glucose broth in order to retain its virulence. Its M.L.D. for mice under these conditions was 0.1 cc of a 24-hour glucose broth culture.

Experiment I—Ninety mice, separated into 3 groups of 30 each, were inoculated intramuscularly with ascending doses of a 24-hour glucose broth culture of Cl. welchii. The first group was injected with 0.05 cc, the second with .075 cc, and the third with 0.1 cc. Seventy-nine of these mice, or 87.7%, died before 72 hours—20 (66%) in the first group, 29 (96.6%) in the second, and all (100%) in the third.

Ninety additional mice were similarly grouped and inoculated, but besides the corresponding dose of the organisms, each was given 1 cc of Neoprontosil intramuscularly at the time of inoculation. Eighty-five (94.4%) mice died before 72 hours—25 (83.3%) in the first group, and all (100%) in the second and third groups (Table I, Exp. I).

Ten mice were similarly inoculated with 1 cc of Neoprontosil only, and all survived.

¹ Bliss, A. L., and Long, P. H., J. Am. Med. Assn., 1937, 109, 1524.

² Carpenter, C. M., and Barbour, G. M., PROC. Soc. EXP. BIOL. AND MED., 1939, 41, 255.

^{*} The Neoprontosil used was 5% solution, kindly supplied by Mr. Rassow, local representative of the Winthrop Chemical Company.

establish the curve of its variation. For this purpose the results recently obtained by McGinty, Anderson and McCullough¹ provide a sensitive qualitative test and within limits (Haskins)² make possible a rough quantitative assay.

Method. Samples of blood were taken every 3 days and the serum extracted with ether. The ether extract was treated with 0.2 N sodium hydroxide and the ether-soluble fraction evaporated and the residue dissolved in peanut oil. A series of dilutions was made in such a way that each 0.1 cc of oil contained the ether-soluble fraction of 1, 2, 3 and 4 cc of normal serum, respectively. The hormone was tested by the method of local intrauterine injections of de Mussio-Fournier, Albrieux, Morato, and Grosso.3 Immature rabbits of an average weight of 710 g were injected daily with 25 I.U. of estrogen (Amniotin) for 6 days. On the 7th day operation was performed under anesthesia, and 2 segments 2 cm long were isolated between ligatures in each uterine horn. Three of these were injected with the dilutions which contained the ether-soluble fraction of the different quantities of normal serum, and the fourth with peanut oil as a control. Autopsies were done at 72 hours after the operation and the segments of the uterus were examined histologically. The degree of endometrial proliferation was evaluated according to the scale of McPhail.4

Results. Figs. 1, 2 and 3 show the results obtained in 3 Rhesus monkeys of the same age and weight. The curve represents the variations of the degree of proliferation of the endometrium of the immature rabbit obtained with 1, 2, 3 and 4 cc, respectively, of blood serum, studied every 3 days. The maximum reactions corresponded to the value †+† on the scale of McPhail and the minimum measured between 0 and †. In 60% of those cases in which the serum extract gave a positive reaction in the segments in which it was injected, the segment injected with peanut oil also showed a positive reaction between ++ to +++ and +, probably because the hormone circulating in the blood stream is concentrated in the peanut oil. For this reason, in the second series the use of peanut oil as a control was abandoned. In the first study, made during the November-

¹ McGinty, D. A., Anderson, L. P., and McCullough, M. B., Endocrinology, 1939, 24, 829.

² Haskins, Arthur L., Jr., Peoc. Soc. Exp. Biol. And Med., 1939, 42, 624.

³ Mussio Fournier, J. C., Albrieux, A. S., Morato, J., and Grosso O., Bull. de l'Acad. de Med. Paris, 1938, 120, 273; Rev. de Obstet. e Ginec. de Sao Paulo, 1938, 3, 203; Endocrinology, 1939, 24, 515.

⁴ McPhail, M. K., J. Physiol., 1935, 83, 145.

the animal succumbed to infection. On examining the lesions produced, one could observe normal phagocytic activity and numerous organisms within the tissue of the lesions produced. Feinstone, Bliss, Ott and Long³ present evidence indicating that the activity of Neoprontosil depends on its reduction to sulfanilamide *in vivo*.

Gye and Cramer⁴ found that ionizable salts of calcium inoculated together with washed spores of *Cl. welchii* or *Cl. tetani* led to the development of the corresponding infections in their fatal form, while washed spores alone did not lead to death. Fildes⁵⁻⁷ thinks that there must be some definite stimulus to vegetation in the tissues injected by calcium salts, and suggests that this stimulus is probably the result of diminished oxygen tension. He showed further that the injection of solutions of calcium chloride lead to the production of localized areas of oxygen deficiency. We do not know if Neoprontosil in this case acts in a similar way.

Summary—We were unable to protect mice from M.L.D. of Cl. welchii by intramuscular injection of Neoprontosil. The intramuscular injection of washed cells of Clostridium welchii with Neoprontosil in mice led to the development of a fatal infection.

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Blood Progesterone During Sexual Cycle of Macaca rhesus; Quantitative Assay.

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Medicine and Dentistry.

At the suggestion of Professor George W. Corner, the presence of progesterone in the blood during the menstrual cycle of the monkey, Macaca rhesus, has been studied and an effort made to

³ Feinstone, W. H., Bliss, E. A., Ott, E., and Long, P. H., Bull. Johns Hopkins Hospital, 1938, 62, 565.

⁴ Gye, W. E., and Cramer, W., Sixth Sci. Rep. Imp. Cancer Res. Fund, 1919, pp. 40-57.

⁵ Fildes, P., Brit. J. Exp. Path., 1927, 8, 387.

⁶ Ibid., 1929, 10, 151.

⁷ Ibid., 1929, 10, 197.

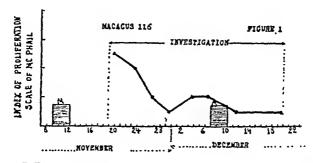
^{*} Fellow of the Argentina Association for the Advancement of Sciences. This work was made possible by a grant made to Dr. George W. Corner through the University of Rochester by the John and Mary R. Markel Foundation.

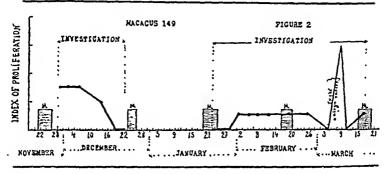
Monkey 163, which showed all negative reactions in the second half of the January-February cycle (Fig. 3) maintained this negativity, during the first and part of that which would have constituted the second part of the February-March cycle, which was incomplete due to the failure of menstruation in the latter month. If we assume that menstruation would have occurred between the third and the seventh of March, the maximum reaction (++) was observed precisely in this period and the curve maintains this maximum during what would have been the first half of the following cycle, thus being in accord with the first study made. In monkey 149 (Fig. 2) weak reactions were obtained at the end of the first and during the second half of the January-February cycle and moderate reactions in the first part of the February-March cycle.

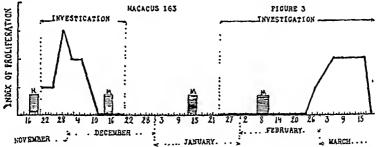
In order to control the procedure used we injected this animal with 0.010 g of progesterone daily for 5 days. Blood taken on the sixth day gave a +++ reaction of proliferation with 2 cc of serum, successive samples being negative.

Quantitative Assay. If we use the figures found by McGinty to evaluate the uterine proliferative reactions we should be able, by trying measured doses, to calculate indirectly the quantity of progesterone existing in the blood at any given moment. If as stated by this author, ++ to +++ reactions in the uterus of the rabbit are produced by doses of progesterone which vary from 5 to 0.5 γ , when we obtain in our case the same reaction with 2 cc of serum, we may deduce that each cubic centimeter of blood serum contained, at the moment it was taken, between 2.5 and 0.25 γ of progesterone.

Conclusions. 1. The presence of progesterone was demonstrated in the blood of the normal Macaca rhesus. 2. The hormone in 1, 2, 3 and 4 cc of blood serum was studied every 3 days by means of local intrauterine injection in immature rabbits and the degrees of progestational reaction of the endometrium measured according to the scale of McPhail. 3. With this procedure it was possible to obtain the curve of its variations during a complete menstrual cycle in 3 Rhesus monkeys of the same age and weight. 4. In 2 of the animals the curve shows its maximum (++ to +++ reaction) on the 10th and 11th day of the menstrual cycle respectively, while in the third it was maintained at a constant level (+ to ++ reaction) during the first half of the cycle. In the 3 monkeys the curve gradually fell during the second half of the menstrual cycle. 5. Studies repeated in 2 of the first animals, during 2 complete cycles, did not give such clear and definite results, perhaps because



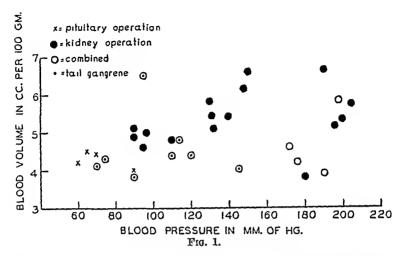




Curves of progesterone in the blood during the sexual cycle of Maeaca rhesus.

December cycle, the curve shows in monkeys 116 and 163 (Fig. 1 and 3) a maximum reaction of +++ and ++ to +++, respectively, on the 10th and 11th day of the cycle, while in monkey 149 (Fig. 2) it is maintained at the same level (+ to ++) during this first half of the cycle. In the 3 animals the curve drops gradually in the second half of the intermenstrual period; but while in 2 of them (Fig. 2 and 3) it becomes negative 3 and 5 days before the next menstruation, in monkey 116 (Fig. 1) it reaches a proliferative reaction equal to + the day before the catamenial flow.

The great similarity of results in this first cycle was not observed when the investigation was repeated in animals 163 and 149.



Blood pressure of the normal rats, under nembutal anesthesia, will not exceed 140 mm of mercury when measured by this method, and less than 5% of the animals will show pressures in excess of 120. Blood volume, expressed in cc per 100 g body weight ranges from 4.0 to 5.0 cc in normal animals weighing over 160 g while the upper limit in animals weighing less than 160 g is 5.3. All animals used in this study weighed well over 160 g.

It is seen in Fig. 1 than in half of the 16 animals, represented by solid dots, the blood pressure was 140 or over. In all rats with blood pressures of 140 or higher the blood volume was 5.1 or more except in one case, a female with a blood pressure of 180 and a blood volume of only 3.8. This animal may have been pregnant and, if so, should not be included in the series. Some elevation of blood volume occurred in 3 animals with blood pressures over 130, while the remaining animals with definitely normal blood pressures tended to have blood volumes within the normal range.

Procedure 2. The posterior lobe and half the anterior lobe of the pituitary were resected in 4 animals by the usual parapharyngeal route. All promptly developed typical diabetes insipidus. Blood pressure and blood volume measurements, made 7 to 10 days later, are charted as crosses in Fig. 1. All were normal.

Procedure 3. Fourteen animals survived the combined pituitary and kidney operations as described in procedures 1 and 2. The operations were performed in 3 stages a week apart, the usual order being subtotal nephrectomy, pituitary operation, nephrectomy. The first blood pressure measurement was made one week after the last

of lesser sensitivity of the rabbits to the same small quantities of progesterone which were found in the first cycle, rather than to a diminution of the circulating hormone. 6. By calculation from the figures obtained by McGinty, we arrive at the conclusion that during the menstrual cycle of one of the animals studied, the amount of progesterone in 1 cc of blood serum at any given moment varied between a maximum of 0.25 to 2.5 γ and a minimum of 0.06 to 0.12 γ .

11519

Blood Volume in Experimental Hypertension Following Subtotal Nephrectomy. Effect of Posterior Pituitary Lobectomy.

J. Q. Griffith, Jr.,* and Dwight J. Ingle.

From the Robinette Foundation, the George S. Cox Medical Research Institute, and the Medical Clinic of the Hospital of the University of Pennsylvania.

Chanutin and Ferris¹ described the development of vascular hypertension in rats after a 2-stage operation in which two-thirds of one kidney and, later, all of the remaining opposite kidney were removed. McQueen-Williams, quoted by Winternitz,² noted that hypertrophy of the fragment of kidney remaining did not occur in such an animal if the pituitary was removed. It was not stated whether the hypophysectomy had any effect upon the development of the vascular hypertension. The following studies were undertaken to determine whether there was any blood volume change associated with an inadequate amount of renal substance, and what relationship the pituitary, especially the posterior lobe, might bear to such change and to the blood pressure.

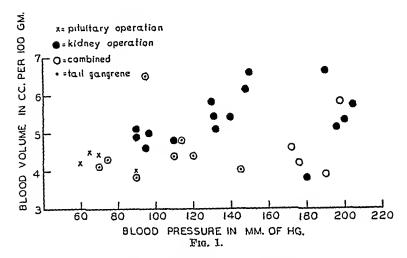
Procedure 1. A series of rats were subjected to the procedure described by Chanutin and Ferris; 16 rats survived. Seven to 10 days later blood pressure was measured by the indirect method of Griffith³ and blood volume by the method of Griffith and Campbell.⁴

^{*} Atwater Kent Fellow in Medicine.

¹ Chanutin, A., and Ferris, E. B., Jr., Arch. Int. Med., 1932, 49, 767.

² McQueen-Williams, M., unpublished. Quoted by Winternitz, M. C., Thomas,
R. M., and LeCompte, P. M., The Biology of Arteriosclerosis, C. C. Thomas, 1938.
3 Griffith, J. Q., Jr., Proc. Soc. Exp. Biol. And Med., 1934, 32, 394.

⁴ Griffith, J. Q., Jr., and Campbell, R., PROC. Soc. Exp. Biol. And Med., 1937, 36, 38.



Blood pressure of the normal rats, under nembutal anesthesia, will not exceed 140 mm of mercury when measured by this method, and less than 5% of the animals will show pressures in excess of 120. Blood volume, expressed in cc per 100 g body weight ranges from 4.0 to 5.0 cc in normal animals weighing over 160 g while the upper limit in animals weighing less than 160 g is 5.3. All animals used in this study weighed well over 160 g.

It is seen in Fig. 1 than in half of the 16 animals, represented by solid dots, the blood pressure was 140 or over. In all rats with blood pressures of 140 or higher the blood volume was 5.1 or more except in one case, a female with a blood pressure of 180 and a blood volume of only 3.8. This animal may have been pregnant and, if so, should not be included in the series. Some elevation of blood volume occurred in 3 animals with blood pressures over 130, while the remaining animals with definitely normal blood pressures tended to have blood volumes within the normal range.

Procedure 2. The posterior lobe and half the anterior lobe of the pituitary were resected in 4 animals by the usual parapharyngeal route. All promptly developed typical diabetes insipidus. Blood pressure and blood volume measurements, made 7 to 10 days later, are charted as crosses in Fig. 1. All were normal.

Procedure 3. Fourteen animals survived the combined pituitary and kidney operations as described in procedures 1 and 2. The operations were performed in 3 stages a week apart, the usual order being subtotal nephrectomy, pituitary operation, nephrectomy. The first blood pressure measurement was made one week after the last

operation. Only 2 of the 14 animals were hypertensive. This measurement was repeated one week later, when 7 out of 14 were found to be hypertensive. Thus the appearance of the hypertension was somewhat delayed. At this time blood volume was measured in 12 animals (one died and in one there was a technical error in the intravenous injection of the dye). The results are shown in Fig. 1 as open circles. Blood volume was increased in only 2 animals, one of which was hypertensive while one was not. Blood volume was normal in 4 animals with hypertension.

As an unexpected finding, certain animals undergoing the combined operations as outlined in procedure 3 developed gangrene of the tail, which may be described as moist in contrast with the dry gangrene seen after ergotamine poisoning. This occurred in 8 of the 13 surviving animals, 12 of which are charted in Fig. 1. Rats with gangrene are indicated by dots placed centrally in the open circles. The 13th animal had a blood pressure of 163, no tail gangrene, but blood volume determination failed and, therefore, it is not charted. It is apparent that there was a tendency for animals developing hypertension to escape the gangrene.

Comment. While the number of animals is too small to permit absolute conclusions, certain tendencies are obvious. The increase in blood pressure occurring in rats with only about half of one kidney remaining is associated, as a rule, with increased blood volume. In the absence of the posterior lobe of the pituitary, such elevation in blood volume does not occur, but the vascular hypertension is unaffected. This may mean that the increased blood volume is maintained through the mediation of the posterior lobe of the pituitary, or, more likely, that in the presence of a marked continuous diuresis an increased blood volume is more difficult to main-The gangrene of the tail is difficult to explain. It has never occurred in several hundred cases where either the kidney or the pituitary operations were performed alone. It would appear that without a vascular hypertension the animal is unable adequately to maintain its peripheral circulation under the conditions detailed in procedure 3.

Summary. Experimental hypertension may develop in the partially nephrectomized rat in either the presence or absence of the posterior pituitary. In the presence of the posterior pituitary 7 of 8 hypertensive rats showed an increase in blood volume whereas in the absence of the posterior pituitary 4 of 5 hypertensive rats showed a normal blood volume.

11520

Axon Branching After Nerve Regeneration.*

W. G. WATROUS. (Introduced by J. M. D. Olmsted.)

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Berkeley, California.

Langley and Anderson¹ proved by physiological methods that regenerating axons often branch; Kilvington² employed both physiological and histological methods to demonstrate the multiplication of axons in the regenerating segment of a peripheral nerve. Bender and Fulton³ showed functional disorders after regeneration of the oculomotor nerve in the chimpanzee and attributed them to fiber splitting and aberrancy. There is complete unanimity regarding the presence of branching axons during regeneration, and in fact this response has been used as a method to make up for the deficit of axons in cases of anterior poliomyelitis (Feiss,⁴ Dogliotti,⁵, Aird and Naffziger,⁴ and others). To our knowledge, however, there have been no experiments reported which directly record those muscular contractions which fiber branching makes possible through the axon-reflex.

After section of the peroneal and popliteal branches of the sciatic nerve at the knee in the cat and dog and regeneration has well commenced, an opportunity is afforded to show the axon-reflex contraction in both the anterior and the posterior tibial muscles. Stimulation of the posterior tibial nerve (a mixed nerve) at the heel will cause a contraction of the gastrocnemius and stimulation of the superficial peroneal nerve (sensory only) at the ankle will provoke a contraction in the tibialis anticus even after cutting the sciatic in the thigh (Fig. 1). With the use of a strong tetanizing current, the curves are similar in form to those produced by stimulating the appropriate motor branch of the sciatic nerve with a like current. Progressive downward section of the sciatic nerve to a point just above the neuroma does not affect the contraction in the least, but

^{*} This investigation has been supported by a grant from the Research Board of the University of California.

¹ Langley, J. N., and Anderson, H. K., J. Physiol., 1902, 29, iii.

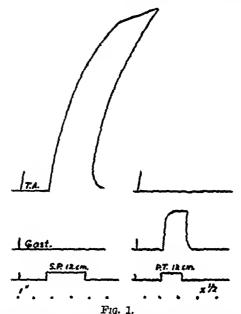
² Kilvington, B., Brit. Med. J., 1905, 1, 935.

³ Bender, M. B., and Fulton, J. F., J. Neurophysiol., 1938, 1, 144.

⁴ Feiss, H. O., Bost. Med. and Sci. J., 1911, 164, 667.

⁵ Dogliotti, A. M., J. dc Chir., 1935, 45, 30.

⁶ Aird, R., and Naffziger, H. C., Arch. Surg., 1939, 38, 906.



Tracing of an axon-reflex contraction of the tibialis anticus on stimulation of the superficial peroneal nerve, and of the gastrocnemius on stimulation of the posterior tibial nerve, after cutting the sciatic in the thigh.

section of the sciatic nerve just below the neuroma immediately and permanently abolishes the axon-reflex contraction. No histological counts were made, but an idea of the degree of fiber-splitting may be gained from the fact that such an axon-reflex contraction may be as much as three-fourths the height of the contraction caused by maximal motor nerve stimulation.

The fiber branching undoubtedly contributes to the incoordination which follows nerve section and nerve regeneration in the limb, as previously shown by Bender and Fulton³ for the eye, but since the peroneal nerve innervates exclusively flexors and the popliteal nerve innervates mainly extensors, this factor is minimized. The axonreflexes furthermore persist and are not eliminated by a process of atrophy as suggested by Langley⁷ for aberrant fibers, since they are found in full force even after eighteen months.

Summary. Confirmation of axon branching as a result of nerve regeneration has been obtained by physiological methods. The axon-reflex contraction made possible through such branching possesses all the characteristics of a muscular contraction evoked by direct electrical stimulation of the motor nerves concerned.

⁷ Langley, J. N., J. Physiol., 1897, 22, 215.

11521

Generalized Edema in Chicks Prevented by d, l-Alpha Tocopherol.*

H. R. BIRD AND THOS. G. CULTON. (Introduced by T. C. Byerly.)

From the Department of Poultry Husbandry of the University of Maryland,

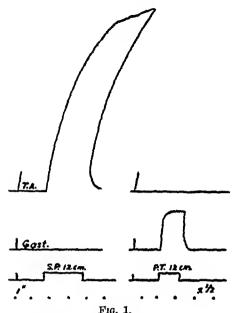
College Park, Maryland.

The experiments here reported were designed to study the nutritive completeness of different samples of dried skimmilk for chicks. A basal ration was developed in which all the protein is supplied by dried skimmilk and the known deficiencies of dried skimmilk are corrected by the addition of small amounts of various supplements. This ration, referred to as Ration 3, has the following composition: dried skimmilk 54%, dextrinized starch 44%, ground limestone 1%, and NaCl 1%, plus 0.12% of ferric citrate, 0.012% of MnSO₄·4H₂O and 0.0012% of CuSO₄·5H₂O. Cod liver oil is administered by pipette thrice weekly. Chicks consume this diet readily but grow at a somewhat subnormal rate.

When certain samples of dried skimmilk are fed as a part of this ration, a severe generalized edema develops in a large percentage of the chicks. The first manifestation of this condition is extreme subcutaneous edema in some chicks as early as 3 weeks of age, and sufficient in extent to be readily detected without handling the chicks. Many chicks develop a characteristic straddling stance, the legs being forced apart by the great accumulation of fluid under the skin of the ventral body surface. The subcutaneous edema may also extend to the neck and back of the head. No recoveries have been observed; al! birds so affected have died within 2 or 3 weeks. Death is frequently preceded by stupor, seldom lasting longer than 10 to 12 hours. Labored breathing is frequently observed during this period.

A considerable proportion of the chicks die between the ages of 3 and 9 weeks without manifesting any subcutaneous edema. The most consistent post mortem finding in such chicks, as well as in those with subcutaneous edema, has been extreme distention of the heart and pericardium, the latter being filled with exudate. Other common post mortem findings have been ascites, edema of the brain and lungs, and coronary and intestinal hyperemia. In one case, in excess of 50 cc of ascitic fluid were removed from a 600 g bird.

^{*} Supported in part by a grant from the American Dry Milk Institute, Inc. Scientific Contribution No. 514, Maryland Agricultural Experiment Station.



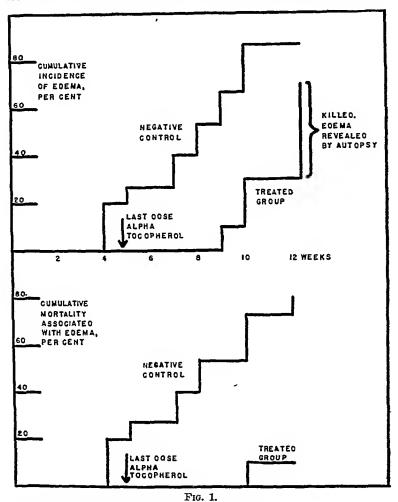
Tracing of an axon-reflex contraction of the tibialis anticus on stimulation of the superficial peroneal nerve, and of the gastrocnemius on stimulation of the posterior tibial nerve, after cutting the sciatic in the thigh.

section of the sciatic nerve just below the neuroma immediately and permanently abolishes the axon-reflex contraction. No histological counts were made, but an idea of the degree of fiber-splitting may be gained from the fact that such an axon-reflex contraction may be as much as three-fourths the height of the contraction caused by maximal motor nerve stimulation.

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⁷ Langley, J. N., J. Physiol., 1897, 22, 215.



Effect of administration of d,l-Alpha Tocopherol on incidence of edema and mortality associated with edema in chicks fed Ration 3.

At this time 10 of the 15 untreated birds had become tocopherol. edematous. The first death associated with edema in the treated group occurred at 10 weeks of age, at which time 11 of the 15 untreated chicks had died with edema. These results would seem to show conclusively that this disease is a manifestation of a deficiency of d,l-alpha tocopherol.

Comparison of the symptoms described here with those described in detail by Dam and Glavind2 would seem to indicate that the differ-

² Dam, H., and Glavind, J., Skand. Arch. fur Physiol., 1939, 82, 299.

Mortality and Incidence of Edema in Chicks Fed Ration 3 with and without Supplements.

Exp.			37 . 6	% edema	% mortality
No.	Skimmilk	Supplement	No. of chicks	5 wk 8 wk	5 wk 8 wk
2	S* W*	0	13 16	62 19	62 31
3	s W	0 0	30 30	37 53 57 77	50 73 67 90
6	s w s w s	0 0 3% dehydrated grass 3% '', 6% yeast 6% '',	33 32 32 32 32 32	30 42 31 53 0 0 0 0 25 50 26 46	51 93 59 94 9 25 3 9 41 72 46 89

^{*} S = Summer; W = Winter.

The first proof that the edema was the result of dietary deficiency was afforded by an experiment in which dehydrated cereal grass; was fed as a supplement to the basal ration. The results of this and other experiments are summarized in Table I. Samples of dried skimmilk prepared in summer and in winter were fed, but no difference in response was observed. The figures in the table establish the protective effect of grass and the lack of protective effect of yeast.

A trial of the effectiveness of d,l-alpha tocopherol was suggested by the report of Dam and Glavind¹ on its effectiveness in preventing the "alimentary exudative diathesis" described by them. Synthetic d,l-alpha tocopherol‡ was dissolved and suitably diluted with cod liver oil and administered thrice weekly to a group of 10 chicks in doses of such size as to approximate 7.5 µg per gram live weight per day. Dosing was begun when the chicks were 4 days of age and continued to the age of 32 days at which time the supply of alpha tocopherol was exhausted. The negative control group consisted of 15 chicks. The results of this experiment are shown graphically in Fig. 1 which shows the cumulative incidence of edema and the cumulative mortality associated with edema. The first case of edema did not appear in the treated group until the chicks were 9 weeks old, 4½ weeks after the administration of the last dose of alpha

[†] Supplied by the American Butter Company, Kansas City, Mo., through the courtesy of Dr. W. R. Graham, Jr.

[‡] Supplied by Merck and Co., Inc., Rahway, N. J., through the courtesy of Dr. G. W. Lewis.

¹ Dam, H., and Glavind, J., Nature, 1939, 143, 810.

guinea pigs and hence may be assumed to exert a destructive effect on vitamin E. Such destruction was not evidenced under the conditions of this experiment, and administration of alpha tocopherol in cod liver oil appears to be an entirely practical procedure in experiments of this kind. Nondestearinated U.S.P. cod liver oil was used, and 100 mg of alpha tocopherol were dissolved and diluted in this oil at one time. Such an amount was sufficient to last for 7 to 10 days. It was kept in the refrigerator when not in use.

Summary. A large percentage of chicks fed a laboratory diet of dried skimmilk, dextrinized corn starch, cod liver oil, and mineral salts develop a generalized edema and die. The most consistent post mortem finding is edema of the heart and pericardium. This disease can be prevented by administration in cod liver oil of synthetic d,l-alpha tocopherol. The disease has not been observed in any chicks fed practical rations.

11522

Effect of Amino Acids, of Vitamin B Complex and Other Compounds on Respiration of Bakers' Yeast.

ELTON S. COOK, ELSIE M. WALTER AND SISTER MARY REDEMPTA EILERT, S.S.J.* (Introduced by S. Tashiro.)

From the Research Laboratories of the Institutum Divi Thomae, Cincinnati, Ohio.

In our laboratories a considerable amount of work has been done on the effects of various fractions from yeast and other sources on the respiration and proliferation of yeast and tissues. In this connection experiments have been performed with pure substances some of which may be present in these preparations. The results of these experiments are the subject of the present paper.

Although we have used pure cultures of yeast in some of our respiration work (pure cultures are always used in proliferation studies), it has been desirable to have a readily available and reasonably constant source of yeast in quantity. We have found that Fleischmann's bakers' yeast answers these requirements fairly well. (Anheuser-Busch bakers' yeast has also been used with equal satisfaction in later work, but the experiments herein reported deal with

^{*} Assistance in some experiments was given by Vincent Sacksteder.

ences are differences of degree. The Danish workers report exudation in subcutaneous tissues and, rarely, in the cavum peritoneum; and they report, further, that the disease as observed by them terminates frequently in recovery and occasionally in death. From this standpoint as well as from the standpoint of ease of preparation, the ration used in these studies would appear to offer greater possibilities for vitamin E assay than the ration used by Dam and Glavind, who have suggested that an assay method might be developed on the basis of their experiments. Further experiments, designed to explore these possibilities, are in progress.

It is of interest to point out that localized edema is consistent and conspicuous among the histopathological changes in the brains of encephalomalacic chicks, as reported by Pappenheimer et al.,3 since Dam et al.4 have found this disease also to be preventable by d,l-alpha tocopherol. It should be pointed out also that Pappenheimer et al. reported a very low incidence of subcutaneous edema in encephalomalacic chicks observed by them. In these experiments edema of the brain was frequently noted, but in no case were the symptoms of encephalomalacia observed in the living edematous birds. Two cases of encephalomalacia were observed among the groups in which generalized edema was prevented by feeding dehydrated grass. This is in agreement with Dam's statement that encephalomalacia occurs on a higher intake of vitamin E than does exudative diathesis.

Dam and Glavind² have pointed out that the edema-producing diets used by them were very low in fat, and have discussed the relationship of this fact to the finding of Pappenheimer et al.³ that the incidence of encephalomalacia was increased by increasing the fat content of the diet. They postulate further that vitamin E may act in two different ways against encephalomalacia and exudative diathesis respectively. It may be noted that the edema-producing diet described in this paper is also low in fat although none of the constituents were subjected to extraction to lower their fat content.

The results of the experiments with alpha tocopherol are of some interest from the standpoint of method of administration since it has been reported by Madsen *et al.*⁵ and by Morris⁶ that cod liver oil favors the development of muscular dystrophy in rabbits and

³ Pappenheimer, A. M., Goettsch, M., and Jungherr, E., Conn. Agr. Exp. Sta. Bul., 229, 1939.

⁴ Dam, H., Glavind, J., Bernth, O., and Hagens, E., Nature, 1938, 142, 1157.

⁵ Madsen, L. L., McCay, C. M., and Maynard, L. A., Proc. Soc. Exp. Biol. and Med., 1933, 30, 1434.

⁶ Morris, S. G., Science, 1939, 90, 424.

guinea pigs and hence may be assumed to exert a destructive effect on vitamin E. Such destruction was not evidenced under the conditions of this experiment, and administration of alpha tocopherol in cod liver oil appears to be an entirely practical procedure in experiments of this kind. Nondestearinated U.S.P. cod liver oil was used, and 100 mg of alpha tocopherol were dissolved and diluted in this oil at one time. Such an amount was sufficient to last for 7 to 10 days. It was kept in the refrigerator when not in use.

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11522

Effect of Amino Acids, of Vitamin B Complex and Other Compounds on Respiration of Bakers' Yeast.

ELTON S. COOK, ELSIE M. WALTER AND SISTER MARY REDEMPTA EILERT, S.S.J.* (Introduced by S. Tashiro.)

From the Research Laboratories of the Institutum Divi Thomae, Cincinnati, Ohio.

In our laboratories a considerable amount of work has been done on the effects of various fractions from yeast and other sources on the respiration and proliferation of yeast and tissues. In this connection experiments have been performed with pure substances some of which may be present in these preparations. The results of these experiments are the subject of the present paper.

Although we have used pure cultures of yeast in some of our respiration work (pure cultures are always used in proliferation studies), it has been desirable to have a readily available and reasonably constant source of yeast in quantity. We have found that Fleischmann's bakers' yeast answers these requirements fairly well. (Anheuser-Busch bakers' yeast has also been used with equal satisfaction in later work, but the experiments herein reported deal with

^{*} Assistance in some experiments was given by Vincent Sacksteder.

Fleischmann's.) To insure sufficient uniformity we always use the yeast within 3 days after the "cutting" date.

All determinations were made by the direct Warburg method, using the technic previously described.1,2 Fleischmann's bakers' yeast from the center of a fresh 1-lb cake was washed 2 times by centrifugation with the suspending medium (Ringer-phosphate-glucose; 0.02% glucose; pH 7.3). The yeast was then made up with fresh medium to give a count of 250 as determined by a photoelectric densitometer. A count of 1 equals 250,000 cells per cc, or 0.064 mg (wet weight) of yeast per cc. One cc of the yeast suspension (containing an average of 3.9 mg dry weight of yeast), the desired amount of test solution and sufficient Ringer-phosphateglucose to give a volume of 3.1 cc were placed in the outer well of the manometer flask and 0.2 cc of N KOH was placed in the inner well (trial showed the use of KOH papers to be unnecessary). After an equilibrium period of 15 minutes, the manometers were shaken for a 25-minute respiration period, in air, at 37.5°C. Under these conditions the control respirations usually ran between 40 and 60 mm³ corresponding to a $Q_{O_2}^{air}$ of about 25 to 37.

The substances tested were usually dissolved, or in some cases suspended, in Ringer-phosphate-glucose; a few of the less soluble materials were dissolved in distilled water. After adjustment of pH to 7.3 with sodium hydroxide or hydrochloric acid, the concentration was such that no more than 1 cc of the solution was added to the flasks. Certain of the substances, normally insoluble, were thus dissolved as the sodium salts.

Variations of $\pm 5.7\%$ are within experimental error. Only stimulations of 10% or greater are regarded as being significant. All data reported are the result of 2 to 6 determinations at each of not fewer than 4 concentration values within the limits given.

Of the amino acids examined in concentration ranges of 0.069 to 1.0 mg per cc, the following were inactive: d-alanine, arginine, cystine, glutamic acid, glycine, l-histidine, dl- β -phenylalanine, l-proline, and l-tryptophane. The following, in different experiments, varied from inactive to slightly active (10-15% stimulation): dl-alanine, l-alanine, β -alanine, l-aspartic acid, l-leucine and tyrosine.

In another series of experiments several members of the vitamin B complex were examined.† Of these, nicotinic acid (0.077-0.77

¹ Cook, E. S., Kreke, C. W., and Nutini, L. G., Studies Inst. Divi Thomae, 1938, 2, 23; Cook, E. S., Hart, M. J., and Joly, R. S., Proc. Soc. Exp. Biol. And Med., 1938, 38, 169.

² Cook, E. S., and Morgan, M. N., Biochem. J., 1940, 34, 15.

	TABLE				
Effect of Thiamin	Hydrochloride on	Respiration	οf	Bakers'	Yeast.

Conc., mg/cc	% stimulation		
.008	8		
.017	6		
.042	12		
.083	26		
.166	26		
.420	34		
.830	0		

mg/cc) and riboflavin (0.0097-0.97 mg/cc) were inactive. Vitamin B_0 (0.01-0.98 mg/cc) was generally inactive although occasional stimulation of the order of 10% was found with some batches of yeast. Thiamin hydrochloride showed an activity dependent upon the concentration, there being an optimum concentration range as shown by the results of a typical experiment in Table I. Of the 2 components of the thiamin molecule, 4-methyl-5- β -hydroxyethyl thiazole showed no significant activity in concentrations of 0.012 to 1.15 mg per cc. 2-Methyl-4-amino-5-ethoxymethyl pyrimidine was inactive between 1.05 and 0.11 mg per cc; slight activity (10-13%) was found in concentrations down to 0.01 mg per cc. However, an equimolecular mixture of the 2 showed the same activity as thiamin in total concentration ranges of 0.05 to 0.50 mg per cc (e.g., at 0.1 mg/cc, thiamin gave 29% and the mixture 30% stimulation).

The following substances were also inactive in stimulating respiration: adenosine phosphate (Hoffmann-LaRoche) (0.076-0.76 mg/cc), yeast nucleic acid (0.08-0.8 mg/cc), thymus nucleic acid (0.08-0.8 mg/cc), inositol (0.097-0.97 mg/cc) and hippuric acid (0.094-0.94 mg/cc).

Insulin (Iletin, Lilly) was inactive in a concentration of 0.016 mg (or 0.13 unit) per cc but caused 18% stimulation in 10 times this concentration. Concentrations of 0.8 mg (6.5 units) and 1.6 mg (13 units) per cc gave, respectively, 240% and 330% stimulation.

From the results it is seen that most of the amino acids, under the conditions used, were inactive or of a very low order of activity. It will be recalled that 1-leucine³ and β -alanine^{3,4} (by itself or com-

t We wish to thank Drs. R. T. Major and J. M. Carlisle of Merck and Co. for gifts of synthetic thiamin, 2-methyl-4-amino-5-ethoxymethyl pyrimidine, 4-methyl-5- β -hydroxyethyl thiazole, riboflavin and vitamin B_{δ} .

³ Miller, W. L., Trans. Roy. Soc. Can., III, 1936, 30, 99.

⁴ Williams, R. J., and Rohrmann, E., J. Am. Chem. Soc., 1936, 58, 695.

Fleischmann's.) To insure sufficient uniformity we always use the yeast within 3 days after the "cutting" date.

All determinations were made by the direct Warburg method, using the technic previously described. 1,2 Fleischmann's bakers' yeast from the center of a fresh 1-lb cake was washed 2 times by centrifugation with the suspending medium (Ringer-phosphate-glucose; 0.02% glucose; pH 7.3). The yeast was then made up with fresh medium to give a count of 250 as determined by a photoelectric densitometer. A count of 1 equals 250,000 cells per cc, or 0.064 mg (wet weight) of yeast per cc. One cc of the yeast suspension (containing an average of 3.9 mg dry weight of yeast), the desired amount of test solution and sufficient Ringer-phosphateglucose to give a volume of 3.1 cc were placed in the outer well of the manometer flask and 0.2 cc of N KOH was placed in the inner well (trial showed the use of KOH papers to be unnecessary). After an equilibrium period of 15 minutes, the manometers were shaken for a 25-minute respiration period, in air, at 37.5°C. Under these conditions the control respirations usually ran between 40 and 60 mm³ corresponding to a $Q_{0_2}^{alr}$ of about 25 to 37.

The substances tested were usually dissolved, or in some cases suspended, in Ringer-phosphate-glucose; a few of the less soluble materials were dissolved in distilled water. After adjustment of pH to 7.3 with sodium hydroxide or hydrochloric acid, the concentration was such that no more than 1 cc of the solution was added to the flasks. Certain of the substances, normally insoluble, were thus dissolved as the sodium salts.

Variations of $\pm 5.7\%$ are within experimental error. Only stimulations of 10% or greater are regarded as being significant. All data reported are the result of 2 to 6 determinations at each of not fewer than 4 concentration values within the limits given.

Of the amino acids examined in concentration ranges of 0.069 to 1.0 mg per cc, the following were inactive: d-alanine, arginine, cystine, glutamic acid, glycine, l-histidine, dl- β -phenylalanine, l-proline, and 1-tryptophane. The following, in different experiments, varied from inactive to slightly active (10-15% stimulation): dl-alanine, l-alanine, β -alanine, l-aspartic acid, l-leucine and tyrosine.

In another series of experiments several members of the vitamin B complex were examined.† Of these, nicotinic acid (0.077-0.77

¹ Cook, E. S., Kreke, C. W., and Nutini, L. G., Studies Inst. Divi Thomae, 1938, 2, 23; Cook, E. S., Hart, M. J., and Joly, R. S., PROC. SOC. EXP. BIOL. AND MED., 1938, 38, 169.

² Cook, E. S., and Morgan, M. N., Biochem. J., 1940, 34, 15.

Obviously, as with growth factors, the effectiveness of various substances on the respiration of yeast will depend upon the strain of yeast, the medium, and the general technique. The present results are of value in checking against the activity of various fractions from yeast and animal tissues which we are assaying by means of the technique used in this paper. Under different conditions certain of the apparently inactive materials may assume importance when they become limiting substances. Suggestions of this are seen in the case of several amino acids and vitamin B₀.

Summary. A number of amino acids, members of the vitamin B complex, and miscellaneous substances have been examined for their effects on the respiration of Fleischmann's bakers' yeast. Most of the substances are inactive or only slightly active, but thiamin, or a mixture of its pyrimidine and thiazole components, and insulin have marked activity.

11523 P

Quantitative Studies of Cell Types in Rat Hypophysis Following Administration of Antigonadotropic Serum.*

> JOHN C. FINERTY, HERBERT S. KUPPERMAN AND ROLAND K. MEYER.

From the Department of Zoology, University of Wisconsin.

Several reports in the literature have shown that treatment of animals with antigonadotropic serum causes a condition in the pituitary gland analogous to that found in castrated animals. Severinghaus and Thompson^{1, 2} have described cytological changes in the hypophyses of dogs injected with antihormones. In these animals there was an increase in the basophile cells and a corresponding decrease in the chromophobes which was associated with an atrophy of the gonads, thyroids, and adrenals. Physiological effects have been demonstrated in rats by Meyer and Kupperman³ who

^{*} Aided in part by a grant from the Wisconsin Alumni Research Foundation and by assistance furnished by the personnel of W.P.A. Official Project No. 65-1-53-2349.

¹ Severinghaus, A. E., and Thompson, K. W., Proc. Soc. Exp. Biol. and Med., 1939, 40, 627.

² Severinghaus, A. E., and Thompson, K. W., Am. J. Path., 1939, 15, 391.

³ Meyer, R. K., and Kupperman, H. S., Proc. Soc. Exp. Biol. and Med., 1939, 42, 285.

bined in pantothenic acid^{5,6}) have been identified as bios components for various yeasts. Pratt and Williams⁷ found that, while both pantothenic acid and β -alanine increased the respiration of deficient Gebrüder Mayer yeast, pantothenic acid was ineffective on Fleischmann's cake yeast, presumably owing to an adequate supply of pantothenic acid in the latter yeast. This checks our observations on the usual inactivity of β -alanine. The relative ineffectiveness of the amino acids contrasts markedly with the stimulating effects of non-toxic concentrations of saturated fatty acids on bakers' yeast; these effects appear to be due to the ability of the yeast to oxidize the fatty acids.²

Among the other substances examined, thiamin, vitamin B_0 , and inositol may also act as bios components for certain strains of yeast. Of these, only thiamin was definitely effective in stimulating the respiration of bakers' yeast. Williams⁷ found similar results with both Gebrüder Mayer and Fleischmann's yeasts. It is of interest that, while the 2 components of the thiamin molecule are essentially inactive by themselves (the pyrimidine portion showing only very slight activity), an equimolecular mixture is as effective as the intact molecule. This suggests an ability of the yeast to combine the thiazole and pyrimidine moieties into the whole molecule which would seem to be essential for an increase in respiration. In contrast, Schultz, Atkin and Frey⁸ found the pyrimidine portion to be equally as effective as thiamin in stimulating fermentation by bakers' yeast, the thiazole portion being ineffective.

The inactivity of yeast nucleic acid checks the observation of Pourbaix⁹ who found that sodium nucleinate has no effect on the normal respiration of yeast; it will, however, restore to normal respiration which has been depressed by styryl 430.

The very marked effect of insulin in increasing the oxygen uptake of yeast is particularly interesting in view of the reports that it does not accelerate the fermentation of glucose by yeast.¹⁰ These experiments are being extended in our laboratories.

⁵ Williams, R. J., and Saunders, D. H., Biochem. J., 1934, 28, 1886.

⁶ Rainbow, C., J. Institute Brewing, 1939, 45, 533; Rainbow, C., and Bishop, L. R., ibid., 1939, 45, 593.

⁷ Pratt. E. F., and Williams, R. J., J. Gen. Physiol., 1939, 22, 637.

⁸ Schultz, A. S., Atkin, L., and Frey, C. N., J. Am. Chem. Soc., 1937, 50, 2457.

⁹ Pourbaix, Y., Compt. rend. soc. biol., 1939, 131, 1306.

¹⁰ Fürth, O., Biochem. Z., 1923, 150, 265; Laufberger, V., Z. ges. exp. Med., 1924, 42, 570; Travell, J. G., and Behre, J. A., Proc. Soc. Exp. Biol. and Med., 1923-4, 21, 478; Euler, H. von, and Myrback, K., Z. physiol. chem., 1925, 150, 1.

types in the pituitary gland and the resulting ovarian weights which follow cessation of the antigonadotropic serum treatment are recorded in Table I.

In rats treated for 10 days with antigonadotropic serum and killed on the day following the last injection, the pituitary glands exhibited a picture of extreme basophilism; 31.9% of all the cells were basophiles, in contrast to 5-10% found in normal animals of the same age. The average percentage of chromophobes in these treated animals was 50, showing a definite decrease from the normal level of 70% found in littermate controls. Since no perceptible variation could be detected in the percentage of acidophiles from the normal level during or after treatment, the increase in the number of basophiles appeared to account for the corresponding decrease in chromophobes. The basophilism produced by the injection of antigonadotropic serum appeared to be the same as that found in castrated rats.

Animals killed at later intervals after cessation of treatment showed a progressive decrease in the percentage of basophiles and a proportionate increase in the percentage of chromophobes until about 15 days after the injections were discontinued. At this time the normal ratio of cell types again prevailed, indicating a change of basophiles to chromophobes. Accompanying the decrease in the basophilic elements of the pituitary gland there was a rapid ovarian growth which approached the normal level between the 3rd and 5th days and continued to increase far beyond normal so that by the 15th day after cessation of treatment an average ovarian weight of 59.7 mg had been attained. The average ovarian weight of littermate controls at this age was 17 mg.

Summary. Treatment with antigonadotropic serum resulted in extreme basophilism of the hypophysis of young female rats. When the treatment was discontinued the percentage of basophiles gradually returned to normal with a corresponding increase in the percentage of chromophobes, and marked increase in the size of the ovaries over that of littermate controls.

found that hypersecretion of the pituitary gonadotropic hormone followed treatment with antigonadotropic serum. This effect was determined by precocious development of the ovaries after discontinuing the injections and by ovarian hypertrophy in female rats in parabiosis with pretreated male or female littermates. The work to be reported here corroborates these findings and describes the changes in the number of cells of each of the types in the pituitary gland of female rats following a short period of antigonadotropic serum treatment.

Experimental procedure. Twenty-one female rats were injected subcutaneously from the 10th to the 20th day of life with 0.5 cc per day, or a total dose of 5 cc of antigonadotropic serum obtained from rabbits which had been injected daily with an aqueous extract of whole dried pituitary gland of sheep for a period of 2 months or longer. The serum was shown to be capable of inhibiting the gonadotropic effects of sheep, rat, and human pituitaries, and of pregnant mare serum and prolan. Three rats were killed on the 1st, 3rd, 5th, 7th, 9th, 12th, and 15th day after discontinuing the treatment. Littermate control rats were autopsied at the same age. Pituitary and ovarian weights with the qualitative ovarian response were noted for both the experimental and control animals. pituitary glands were serially sectioned at 6 microns after fixation in Bouin's fluid and stained with a modification of Mallory's trichrome stain (Rasmussen⁴). Three horizontal sections from equidistant levels in the gland were studied in each animal. of these sections all the cells in every tenth field were differentially counted. An average of 2800 cells was counted in each gland.

Results and discussion The changes in the percentages of cell

TABLE I. Ovarian Weights and Percentage of Pituitary Cell Types After Antigonadotropic Treatment.

	7	Avg ovarian	Avg % of pituitary cell types			
Group*	Day killed	wt (mg)	Basophiles	Acidophiles	Chromophobes	
1	20	5.0	31.9	17.3	50.8	
$\bar{2}$	22	7.5	27.4	17.2	53.9	
3	24	20.0	20.8	18.0	61.4	
4	26	20.0	18.3	18.9	62.8	
ริ์	28	20.7	14.8	18.3	6 6.9	
6	31	30.3	12.4	20.3	67.2	
7	34	59.7	9.3	19.4	71.3	

^{*}Each group was comprised of 3 rats which were injected from the 10th to

⁴ Rasmussen, A. T., Am. J. Anat., 1930, 46, 461.

It is well known that the use of excess quantities of an anticoagulant such as sodium oxalate interferes with certain analytical procedures such as deproteinization. Folin-Wu filtrates were made on the samples but no interference with successful deproteinization was detected at any of the concentrations used.

These experiments indicate that sodium hexametaphosphate can be added to the list of blood anticoagulants, and may have certain advantages over existing agents.

11525 P

Barium in the Mammalian Retina.*

GORDON H. SCOTT AND BRUCE CANAGA, JR.

From the Department of Anatomy, Washington University School of Medicine, St. Louis.

Ramage and Sheldon¹ discovered the existence of Ba in the chorioid of ox eyes. They believed that Ba increased in quantity with age and report that it is not present in calves' eyes in sufficient quantity to be detected by their method of flame excitation of the spectrum. Furthermore they say that Ba is not present in the chorioids of human, sheep, pigs, horses, dogs and many sea fish. Ramage and Sheldon failed to find this element in the retina although they could detect it in the iris and the pigment of the chorioid. It is of some significance that they found Ba in the chorioids of all neat cattle beyond 3 years in age. Gerlach and Müller² examined eyes from a wide variety of animals including man and discovered Ba in the chorioid of most of them. It was not present uniformly in human chorioids and there appear to be no age peculiarities in its distribution. These writers also describe Ba in the retinæ of ostrichs, rabbits, cats, cattle and in one human.

The material in the present series consisted of 19 pigs, 17 ox, 24 sheep and 12 kitten eyes. The spectrographic method used was that described by us in an earlier paper (Scott and Canaga³). We used as identifying lines the 4535.5 and 4934.1 Å. These lines are quite sensitive and can be definitely located with little trouble. An

^{*} Aided by a grant from the Josiah Macy, Jr., Foundation.

¹ Ramage and Sheldon, Nature, 1931, 128, 376.

² Gerlach and Müller, Arch. f. path. Anat. u. Physiol., 1936, 296, 558.

³ Scott and Canaga, PROC. Soc. Exp. Biol. AND MED., 1939, 40, 275.

11524 P

Use of Sodium Hexametaphosphate as an Anticoagulant.

CLARENCE E. LARSON.

From the Chemistry Department, College of the Pacific.

Sodium hexametaphosphate has recently become established as an effective agent in reducing calcium ion concentration. Most of the research work on the compound has been done in the field of industrial chemistry where it has demonstrated its use as an outstanding water softener.

In this report we are concerned with the mechanism by which this compound reduces calcium ion concentration. The consensus seems to favor the formula $(N_aPO_3)_6$ or $Na_6P_6O_{18}$. This compound can react with calcium ion to form $Na_2Ca_2P_6O_{18}$ thus leaving the calcium in the form of a complex since the compound ionizes into 2 sodium ions and the negatively charged $Ca_2P_6O_{18}$ ion. The dissociation of this complex ion to yield calcium ion is so slight that the addition of sodium hexametaphosphate depresses the calcium ion to such an extent that it will dissolve the most insoluble calcium salts such as the carbonate, the oxalate, and the phosphate. At present it is impossible to express mathematically the equilibrium constant for the dissociation of the complex ion but indications are that its effective value approaches 10^{-15} .

Comparatively little work has been done on the biochemical and physiological properties of the compound. This paper represents the first report of results of work in progress in our laboratories on the application of properties of sodium hexametaphosphate to the fields of biology and medicine.

The effect of this compound on the clotting of blood was tested by the following method. A stock solution of 20% sodium hexametaphosphate was made up. Varying quantities were added to calibrated tubes so that the final concentrations after blood was added ranged from 0.1 g per 100 cc to 2.0 g per 100 cc. Blood was allowed to flow directly from the sheep being used into the tubes. Each tube was rocked carefully to insure complete mixing. A control tube containing no reagent was treated in the same way. The control tube showed clotting in 5 minutes. All tubes containing sodium hexametaphosphate remained unclotted. Experiments were also performed on human blood with the same results. Concentrations of sodium hexametaphosphate less than 0.1 g per 100 cc slowed the process of clotting but did not prevent it entirely as was the case of the higher concentrations.

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11526 P

Mean Molecular Weights of Synthetic Mixtures of Bovine Plasma Albumin and Globulin.

HENRY L. TAYLOR AND ANCEL KEYS

From the Laboratory of Physiological Hygiene, University of Minnesota, Minneapolis, Minn.

Albumin and globulin, though generally considered to be separate entities, show sedimentation anomalies when they are studied in mixtures with the ultracentrifuge (Pederson¹). The data of Mc-Farlane² suggest that globulin may dissociate in such mixtures. We have studied purified bovine plasma albumin and globulin, alone and in synthetic mixtures, by osmometric methods.

Sterile bovine plasma was carefully fractionated with ammonium sulfate at 0°C by a technic similar to that used by McMeekin.³ The albumin and globulin fractions were reprecipitated, freed of ammonia by dialysis and then dialyzed to equilibrium with phosphate buffer of pH 7.4, $\mu = 0.16$. Osmotic pressures were measured at 0°C by the method to be described by Keys. Protein concentrations were determined with the Pulfrich refractometer in every sample and checked by the micro-Kjeldahl method of Keys⁴ in about every third sample. Mean molecular weights were determined by the method of Adair and Robinson⁵ in which the pressure-concentration ratio is extrapolated at zero concentration. At least 4 acceptable P/C values at below 3% protein concentration were obtained for each sample.

The molecular weights of the albumin and globulin used here were 69,900 and 170,000, respectively, and these values were used to compute the theoretical mean mol. wts. of the mixtures from Dalton's law of partial pressures. The observed mol. wts. of the mixtures deviated widely and systematically from the simple predictions. As shown in Fig. 1, the mean mol. wts. of the mixtures are lower, and the osmotic pressures are higher than predicted. The deviation approaches a linear function of the mol. wt. in the range of A/G from 0.1 to 2.1. Similar results were obtained with other preparations.

¹ Pederson, K. O., Compt. Rend. Lab. Carlsberg, Copenhagen, 1938, 22, 426.

² McFarlane, A. S., Biochem. J., 1935, 29, 407.

³ McMeekin, T. L., J. Am. Chem. Soc., 1939, 61, 2884.

⁴ Keys, A., J. Biol. Chem., 1940, 132, 181.

⁵ Adair, G. S., and Robinson, M. E., Biochem. J., 1930, 24, 1864.

attempt was made to localize Ba in the tissue by separating the retina into 2 layers. Experiments showed that the retinæ split easily along the internal limiting membrane giving one layer which consisted largely of nerve fibers and another which was made up of pigmented epithelium, rods and cones and their nuclei, neuroglial and bipolar cells.

Barium was observed in all specimens examined and was apparently evenly distributed throughout the substance of the retina at least insofar as our rough localization permitted us to estimate. The presence of pigment in the one layer apparently had little or nothing to do with the amount of Ba found. Previously mentioned work seemed to suggest that Ba was associated with the pigment. Spectrographic studies indicate that Ba is not found in any appreciable quantities in other tissues, certainly not in the quantities observable in the retina. Our own experience with some hundreds of samples supports this statement. In fact only one instance is on record in our files and that is the consistent finding of Ba in skeletal muscle the nerve supply of which had been severed 6 months before the examinations were made. In these samples only traces of Ba could be found.

Since a large number of our series consisted of retinæ from neat cattle it is of some interest that Blumberg and Rask⁴ found traces of Ba in milk. However, Ramage and Sheldon state that they have been advised that Ba is more apt to be found in milk which has soured in glass containers. We do not attach much significance to this in relation to Blumberg and Rask's findings as they used pyrex glassware throughout. Our experiments involved digesting the retinæ and other tissues in nitric acid in pyrex vessels. Only the retinæ showed Ba in their spectra. Evidently, therefore, an element, quite active photoelectrically, is present in tissue wherein light is translated into nerve impulses.

⁴ Blumberg and Rask, Nutrition, 1933, 6, 285.

11526 P

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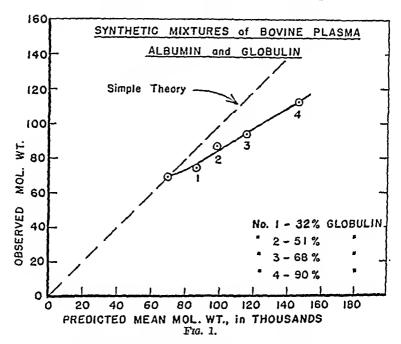
¹ Pederson, K. O., Compt. Rend. Lab. Carlsberg, Copenhagen, 1938, 222, 426.

² McFarlane, A. S., Biochem. J., 1935, 29, 407.

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⁴ Keys, A., J. Biol. Chem., 1940, 132, 131.

⁵ Adair, G. S., and Robinson, M. E., Biochem. J., 1930, 24, 1864.



Sedimentation diagrams of such mixtures clearly show 2 fractions corresponding in molecular size with albumin and globulin but McFarlane's (op. cit.) data on horse plasma proteins indicate that the proportion of the smaller molecules is higher than the composition of his mixtures would warrant. This strongly reinforces our conclusion that globulin dissociates in the presence of albumin. Assuming that the dissociation product is a particle like albumin in size, our results would indicate that about 35% of the globulin undergoes dissociation; in other words, in an albumin-globulin mixture, for every globulin 170,000 molecule, there would be a globulin 70,000 molecule. Alternatively, globulin 170,000 may split into equal halves (mol. wt. 85,000, cf. McFarlane, op. cit.).

These results explain the divergent values reported for the mol. wt. of globulin—145,000 to 192,000. The presence of minute amounts of albumin depresses the mean mol. wt. to a remarkable degree. It is not easy to separate globulin cleanly from albumin; on the other hand, we have had no difficulty in preparing pure albumin. It appears that the formation of dissociated globulin is reversible, that the dissociated globulin salts out with the other globulin and it becomes associated when it enters an albumin-free phase. These phenomena are most striking at very low A/G ratios and are seen in purest form at infinite dilution, but they are de-

monstrable at an A/G ratio of 2.1 and protein concentrations of over 3%. In human plasma (A/G = 2, tot. prot. = 7%) the effect is sufficiently obscured so that the simple relation of Keys⁸ holds approximately.

11527 P

Skin Sensitization to a Simple Compound by Injections of Conjugates.

KARL LANDSTEINER AND MERRILL W. CHASE.

From the Laboratories of the Rockefeller Institute for Medical Research.

In view of a recent paper¹ touching upon the subject, we wish to make a preliminary communication of a study under way for some time on the possibility of producing in animals skin sensitivity to drugs by immunizing with conjugates. We have in fact been able to render guinea pigs sensitive to superficial application of picryl chloride^{2, 3} by intraperitoneal injections of a conjugate resulting from the treatment of guinea pig erythrocyte stromata with picryl chloride in alkaline solution, killed tubercle bacilli as in previous work⁴ having been injected beforehand.

Since even minute quantities of the simple substance can sensitize under certain conditions and must be avoided, the chief concern in these experiments was to guard against the inclusion of unchanged picry! chloride in the injection material. This was carried out by adding an excess of glycine which removed any possible remainder of the substance through chemical combination, and by washing with aqueous alcohol.

The large majority of animals treated in this way have shown upon subsequent testing with the simple substance typical reactions of the contact dermatitis type.

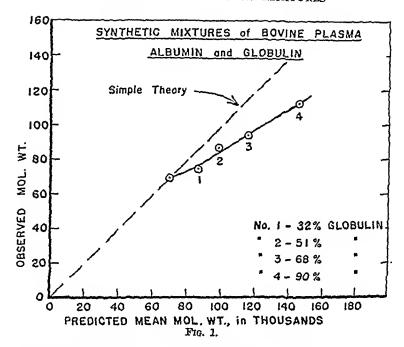
⁶ Keys, A., J. Phys. Chem., 1938, 42, 11.

¹ Haxthausen, H., Acta Derm.-Vener., 1940, 21, 158.

² Landsteiner, K., and Jacobs, J., J. Exp. Med., 1935, 61, 643.

³ Landsteiner, K., and Chase, M. W., J. Exp. Med., 1937, 66, 337.

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³ Landsteiner, K., and Chase, M. W., J. Exp. Med., 1937, 66, 337.

⁴ Landsteiner, K., and Chase, M. W., J. Exp. Med., 1940, 71, 237.

11528

Osmotic Activity Changes of Serum and Salt Solutions Placed in the Gall Bladder.

JOHN W. SCHULZE AND M. B. VISSCHER.

From the Department of Physiology, University of Minnesota.

Dreser,1 the first to use cryoscopy on animal fluids, made the first determination of the osmotic pressure of bile. Numerous early investigators, among them Brand,2 Strauss,3 Bernstein,4 Bosquet,5 Koziezkowsky, Messadaglia and Colletti determined cryoscopically the osmotic pressure of animal and human bile obtained by various methods from living and dead specimens. They came to the conclusion that the osmotic pressure of bile, both bladder and hepatic, was approximately the same as the osmotic pressure of the blood of the same animal species; i.e. the depression of freezing point of the bile and blood both lay in the same range (about -.54°C to -.58°C for most species). Of the many objections to the earlier work, the varied methods of collection and the inaccuracy of the cryoscopic technic are perhaps the most significant. A difference in freezing point of .01°C corresponds to a difference in osmotic pressure of nearly 100 mm of Hg. Ravdin, et al., state that despite the wide variance of constituents, the osmotic pressures of hepatic and bladder bile, as determined by the depression of freezing point, are approximately the same; and that the total depression of freezing point may be accounted for on the basis of the osmolar concentration of base, chloride, and bicarbonate present. Yet the difference in osmotic pressure of their hepatic and bladder bile amounts to 357.2 mm of Hg. They also conclude that the osmotic pressure of hepatic and bladder bile is approximately the same as that of serum. On the hasis of experiments in which they placed various bile constituents individually into a bile-free dog's gall bladder, Ravdin et al.9 came to the conclusion that regardless of the concentration of the original solution, the total osmolar concentration of the fluid in the gall

¹ Dreser, Arch. f. exp. Path. u. Pharm., 1892, 29, 303.

² Brand, Arch. f. d. gesammte Physiol., 1902, 90, 491.

³ Strauss, Berl. Klin. Wehnsch., 1903, 40, 261.

⁴ Bernstein, Arch. f. d. gesammte Physiol., 1905, 109, 207.

⁵ Bosquet, cited by Strauss.

⁶ Koziezkowsky, cited by Strauss.

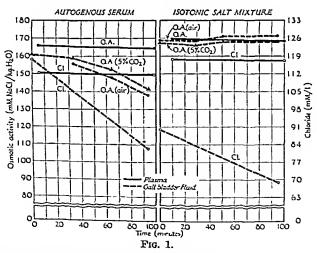
⁷ Messadaglia and Colletti, cited by Strauss.

⁸ Ravdin, Johnston, Riegel and Wright, Am. J. Phys., 1932, 100, 317.

⁹ Ravdin, Johnston, Austin and Riegel, Am. J. Phys., 1932, 99, 638.

bladder after a period of time approaches that of serum. Reinhold and Wilson¹⁰ state that: "Although the sum of the molar concentrations of anions and cations in bile exceeds that in serum, the osmotic pressure of the two fluids, as shown by the work of others, is practically the same. Actually the molar concentrations of inorganic ions are approximately the same in both. It would appear, therefore, that the principal organic ion of dog bile, taurocholic acid, either exhibits little osmotic activity or diminishes the osmotic activity of other ions." Gilman and Cowgill11 using Hill's method found approximate isotonicity of blood and hepatic bile, and that artificially produced changes in the osmotic pressure of the blood produced parallel changes in the osmotic pressure of hepatic bile. Their osmotic pressure values are given in terms of milliequivalents of an osmotically equal NaCl solution. They state they are "confident the values for osmotic pressure—are accurate to within 1 milliequivalent;" yet their average values for the osmotic pressure of blood and hepatic bile under all conditions are 155 and 151 milliequivalents, respectively. Therefore the difference in the average osmotic pressure of blood and bile falls 3 milliequivalents outside the range of experimental error. Furthermore, the values for hepatic bile were 2-9 milliequivalents lower than for blood in 13 of 15

OSMOTIC ACTIVITY AND CHLORIDE CONCENTRATION CHANGES IN THE GALL BLADDER



¹⁰ Reinhold and Wilson, Am. J. Phys., 1934, 107, 378.

¹¹ Gilman and Cowgill, .tm. J. Phys., 1933, 104, 476.

11528

Osmotic Activity Changes of Serum and Salt Solutions Placed in the Gall Bladder.

JOHN W. SCHULZE AND M. B. VISSCHER.

From the Department of Physiology, University of Minnesota.

Dreser,1 the first to use cryoscopy on animal fluids, made the first determination of the osmotic pressure of bile. Numerous early investigators, among them Brand,2 Strauss,3 Bernstein,4 Bosquet,5 Koziezkowsky,6 Messadaglia and Colletti7 determined cryoscopically the osmotic pressure of animal and human bile obtained by various methods from living and dead specimens. They came to the conclusion that the osmotic pressure of bile, both bladder and hepatic, was approximately the same as the osmotic pressure of the blood of the same animal species; i.e. the depression of freezing point of the bile and blood both lay in the same range (about -.54°C to -.58°C for most species). Of the many objections to the earlier work, the varied methods of collection and the inaccuracy of the cryoscopic technic are perhaps the most significant. A difference in freezing point of .01°C corresponds to a difference in osmotic pressure of nearly 100 mm of Hg. Ravdin, et al., state that despite the wide variance of constituents, the osmotic pressures of hepatic and bladder bile, as determined by the depression of freezing point, are approximately the same; and that the total depression of freezing point may be accounted for on the basis of the osmolar concentration of base, chloride, and bicarbonate present. Yet the difference in osmotic pressure of their hepatic and bladder bile amounts to 357.2 mm of Hg. They also conclude that the osmotic pressure of hepatic and bladder bile is approximately the same as that of serum. On the basis of experiments in which they placed various bile constituents individually into a bile-free dog's gall bladder, Ravdin et al.9 came to the conclusion that regardless of the concentration of the original solution, the total osmolar concentration of the fluid in the gall

¹ Dreser, Arch. f. exp. Path. u. Pharm., 1892, 29, 303.

² Brand, Arch. f. d. gesammte Physiol., 1902, 90, 491.

³ Strauss, Berl. Klin. Wehnsch., 1903, 40, 261.

⁴ Bernstein, Arch. f. d. gesammte Physiol., 1905, 109, 207.

⁵ Bosquet, cited by Strauss.

⁶ Koziezkowsky, cited by Strauss.

⁷ Messadaglia and Colletti, cited by Strauss.

⁸ Ravdin, Johnston, Riegel and Wright, Am. J. Phys., 1932, 100, 317.

⁹ Raydin, Johnston, Austin and Riegel, Am. J. Phys., 1932, 99, 638.

15%), and the osmotic activity does not change. The osmotic activity and chloride concentration of blood again remain nearly constant.

Fig. also shows the results of an experiment in which 1 cc of an isotonic mixture of osmotically equal parts of NaCl + Na₂SO₄ was placed in a cat's gall bladder. The volume decreased (50% to 75%). The chloride (25% to 50%). The osmotic activity usually rose somewhat (3 to 5 mM), and was higher in air than in 5% CO₂. The osmotic activity and chloride concentration of the blood remained relatively constant. If the salt solutions are markedly hyper- or hypotonic to the blood (8 to 10 mM), the osmotic activity of the gall bladder fluid decreases or increases, respectively, to approach that of blood. In these cases there is also a decrease in volume and chloride. If the salt solution is poisoned with .001 M HgCl₂; the volume increases (50%), the chloride increases (30%), and the osmotic activity increases more rapidly and markedly (8 mM). The osmotic activity of the plasma remains unchanged.

The osmotic activity of the removed gall bladder bile was usually 1 to 3 mM lower than that of blood removed 15 to 30 minutes later.

11529 P

Cultivation of the St. Louis Encephalitis Virus.*

ELEANORA MOLLOY. (Introduced by C. W. Jungeblut.)

From the Department of Bacteriology. College of Physicians and Surgeons,

Columbia University, New York.

The virus of St. Louis encephalitis is known to grow readily on the chorioallantoic membrane of the developing hen's egg and in tissue cultures of the Li and Rivers type. In both media, however, virus titrations have been uniformly low, usually attaining levels of 10^{-2} , with an occasional maximum of 10^{-3} .

Successful propagation of the lymphogranulonia venereum virus

^{*} This research was supported by a grant from the W. J. Matheson Fund for the study of encephalitis.

¹ Syverton, J. T., and Berry, G. P., Science, 1935, 82, 596.

² Harrison, R. W., and Moore, E., PEOC. Soc. Exp. Biol. And Med., 1936, 35, 359.

¹ Schultz, E. W., Williams, G. F., and Hetherington, A., Proc. Soc. Exp. B10L, AND Med., 1938, 38, 799.

⁴ Smith, M. G., and Lennette, E. H., PROC. Soc. EXP BIOL AND MED., 1939, 41, 323.

cases. In the other 2 cases the value for bile was 1 milliequivalent higher than for blood. In order to investigate the role of the gall bladder in osmotic processes, it was decided to study the changes in osmotic activity of serum and nearly isotonic NaCl + NaSO₄ solutions placed in the gall bladder by the vapor tension method.

Methods. Cats anesthetized with nembutal were used as experimental animals. The gall bladder was entered via a whistle-tip fiber ureteral catheter so inserted and tied into the cystic duct as not to injure the cystic vessels. Injections into and withdrawals from the gall bladder were then accomplished by a syringe whose needle fit closely into the open end of the catheter. When not in use, the open end of the catheter was closed by a piece of wire of similar diameter to the bore of the catheter. After the bladder bile was removed, the gall bladder was washed out several times with normal saline at body temperature then with the fluid to be injected at body temperature. Finally such an amount of fluid was injected as to moderately distend the gall bladder (usually 1.5 to 0.7 cc). The fluids injected were: (1) the cat's own serum obtained just before injection from femoral vein blood, and (2) NaCl + Na₂SO₄ (in about equiosmotic proportions) solution approximately isotonic with the cat's plasma. Small samples of gall bladder fluid were withdrawn about every ½ hour for 1½ to 2 hours. Blood plasma samples were taken as described by Roepke and Visscher12 at the beginning and end of the experiment. All samples were protected from evaporation and CO. loss. Osmotic activity (see Roepke and Visscher¹²) determinations were made with Hill's thermoelectric method as modified by Baldes. 13,14 Vapor tension measurements were made with air and CO2 mixtures in the thermocouple chamber in order to control the influence of the CO2 tension. Chloride was determined according to the method of Van Slyke.15

Results. Fig. 1 shows the typical results obtained on placing 1 cc of autogenous serum in a cat's gall bladder. Within 90 minutes the volume decreases (50% to nearly 100%); the chloride falls (30% to 40%); and the osmotic activity decreases (7 to 23 mM). The osmotic activity is higher in 5% CO₂ than in air. The osmotic activity and chloride concentration of the blood remain relatively constant. If the serum is poisoned with .004 M HgCl₂; the volume remains practically constant, the chloride decreases (but only about

¹² Roepke and Visscher, Proc. Soc. Exp. Biol. and Med., 1939, 41, 500.

¹³ Baldes, J. Sc. Instruments, 1934, 11, 223.

¹⁴ Baldes and Johnson, Biodynamics, 1939, No. 47, 1.

¹⁵ Van Slyke, J. Biol. Chem., 1923, 58, 523.

advantages offered by the chorioallantoic membrane of the developing egg. The chorioallantoic membrane of a 10-day-old egg was removed, washed in saline, and placed in 10 cc of serum ultrafiltrate in a 50 cc Erlenmeyer flask. The cultures were incubated at room temperature and the supernatant fluid only was used for passage and titration. The virus titered up to 10⁻¹ after six passages and up to 10⁻¹ after nine and ten generations. With this type of culture, only very fresh preparations were used, as, on storage, acid accumulates which must be neutralized by alkali in order to maintain a constant pH over a long period of time. The standardization of this type of culture is under consideration at present.

Conclusions. The virus of St. Louis encephalitis may be grown in a medium containing embryonic mouse or guinea pig brain in ox serum ultrafiltrate. Cultures of organs from adult mice fail to support growth of the virus. Incubation at room temperature produces higher titers (10⁻⁵) than incubation at 37⁻²C (10⁻³). At both temperatures the attained titer remains almost unchanged for 10 days but shows a decrease after 15 days' incubation. The virus is present in the same concentration in the supernatant fluid as in the emulsified whole culture. Infected chorioallantoic membranes maintained in serum ultrafiltrate at room temperature support growth of this virus up to titers varying from 10⁻⁴ to 10⁻⁵.

11530

Search for Microörganisms of the Pleuropneumonia Group in Rheumatic and Non-Rheumatic Children.

ALBERT B. SABIN AND BARBARA JOHNSON

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine.

It has recently been demonstrated that mice of various stocks are carriers of a new group of filtrable microörganisms which biologically can be classed with the causative agent of pleuropneumonia botum but otherwise is quite distinct as regards pathogenicity, affinities for special cell types in vico, and immunological identity. In mice, these microörganisms are usually found in association with the epithelium of the conjunctiva and nasal mucosa without giving rise to any signs of disease. However, when cultures of certain

¹ Sabin, A. B., Science, 1939, 90, 18.

was obtained by Sanders⁵ with the use of a new type of medium consisting of tissue elements in ox serum ultrafiltrate.⁶ Higher yields of virus were obtained at room temperature than at 37°C. The stability of these cultures and their marked potency, together with the fact that serum ultrafiltrate is protein-free made it appear desirable to apply this method to the propagation of St. Louis encephalitis virus.

Tissue cultures were prepared in rubber stoppered 50 cc Erlenmeyer flasks by adding minced embryonic mouse brain to 10 cc of serum ultrafiltrate diluted 1 in 3 with Simms' salt solution. These flasks were inoculated with 0.1 cc of a 1:10 mouse brain virus suspension; and parallel series maintained, one at room temperature and one at 37°C. Passages were made by transferring 0.1 cc of the clear supernatant fluid every 5 days. After intervals of 5, 10 and 15 days' incubation, potency tests were carried out by intracerebral inoculation of groups of 4 mice (8-12 g) with 0.03 cc of serial tenfold dilutions of the supernatant fluid. The endpoint in these titrations was taken as the last dilution causing characteristic symptoms and death in 50% of the inoculated mice. The identity of the virus was assured by two neutralization tests with a known antiserum, carried out with the 5th and 28th culture passages.

During 28 culture generations, the virus titers have been consistently higher after incubation at room temperature (10-5) than at 37°C (10-3). These titers, once attained, were maintained at a constant level during the first 10 days, but showed a drop in potency to 10-1, 10-2 after 15 days. No difference was observed between the virus content of the whole culture (emulsified tissue plus supernatant fluid) and that of the clear supernatant fluid alone.

When embryonic guinea pig brain was used in place of mouse brain the virus grew readily at room temperature (10⁻², 10⁻³), but showed a tendency to die out after 4 to 7 passages in cultures of other organs (liver, lung, spleen, kidney, heart, intestine). After 12 passages through embryonic guinea pig brain cultures the virus still failed to infect guinea pigs.

Cultures of adult mouse organs (brain, liver, kidney, spleen, heart, adrenal) have uniformly failed to support the growth of this virus in repeated tests, irrespective of variations in technic, such as temperature, amount of tissue, amount of fluid, method of transfer.

An attempt was made to combine the tissue culture technic with

⁵ Sanders, M., J. Exp. Med., 1940, 71, 113.

⁶ Simms, H. S., and Stillman, N. C., J. Gen. Physiol., 1937, 20, 603.

t The strain used was isolated in 1933 by Dr. M. Holden of this Department.

advantages offered by the chorioallantoic membrane of the developing egg. The chorioallantoic membrane of a 10-day-old egg was removed, washed in saline, and placed in 10 cc of serum ultrafiltrate in a 50 cc Erlenmeyer flask. The cultures were incubated at room temperature and the supernatant fluid only was used for passage and titration. The virus titered up to 10⁻⁴ after six passages and up to 10⁻⁵ after nine and ten generations. With this type of culture, only very fresh preparations were used. as, on storage, acid accumulates which must be neutralized by alkali in order to maintain a constant pH over a long period of time. The standardization of this type of culture is under consideration at present.

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¹ Sabin, A. B., Science, 1939, 90, 18.

types of these microörganisms are injected intravenously or by certain other routes, experimental diseases are produced in mice which resemble in many respects some of the manifestations of rheumatic fever and rheumatoid arthritis in man.2 Many attempts have been made, therefore, to isolate similar microörganisms from these human diseases. Failure to obtain such microörganisms from the exudates and tissues of a small number of patients with rheumatoid arthritis or rheumatic fever has already been reported1-3 and the purpose of the present investigation was (a) to determine whether or not human beings may be carriers of similar or related microörganisms, and (b) to study additional material from patients with rheumatic fever or rheumatoid arthritis

Cultures were obtained from the nose and throat and in most instances also from the conjunctiva of 100 human beings, 95 of whom were under 15 years of age. Material obtained with sterile cotton swabs was streaked on agar plates containing 30% ascitic fluid. After 4 days' and again after 7 days' incubation at 37°C, all plates were examined with the microscope at a magnification of 100 times and a thorough search was made for colonies which might resemble even remotely those of the pleuropneumonia group. No such colonies, however, were found in any of the cultures. Among the patients that were examined in this manner there were 28 in the active phase of rheumatic fever, 2 in the active stage of Still's disease, 14 with various types of infection of the upper respiratory tract (mostly pharyngitis or tonsillitis associated with otitis media), 3 with pneumonia, and most of the remainder with miscellaneous medical or surgical conditions.

The blood of 9 children with rheumatic fever (acute febrile phase) and of two children during the febrile stage of Still's disease was cultured by adding 5 cc to 25 cc of broth containing 30% ascitic fluid and 0.5% glucose. No growth was obtained despite prolonged incubation and "blind passage." Exudates from the knee-joints of 3 children during their first attack of rheumatic polyarthritis were similarly cultured on fluid and solid media, but without obtaining any growth. Pericardial fluid, the pericardium and myocardium, and vegetations from the mitral valve obtained at necropsy from two children who died with active rheumatic carditis also vielded no growth.

Because experience with the pleuropneumonia group in mice indicated that they may often be intimately associated with the affected

<sup>Sabin, A. B., Science, 1938, 88, 575; ibid., 1939, 89, 228.
Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., Brit. J. Exp. Path.,</sup> 1940, 21, 13.

cells, and since the carrier state in mice was established by streaking the conjunctiva and nasal mucosa rather than exudates from those sites, it was decided to investigate a series of tonsils removed from children for various reasons. A piece of tissue was removed from each tonsil, minced to expose a larger surface, and streaked on a 30% ascitic fluid agar plate. The tonsils of 58 children (116 specimens) were thus examined, and in 3 cases there were colonies, 20 to 40 μ in size, bearing a striking resemblance to those of certain members of the pleuropneumonia group. The appearance of these colonies (to be referred to as "X" colonies) is illustrated in Figs. 1 and 2. They always occurred along the streak either independently of the adjacent bacterial colonies (Figs. 1, 2) or at the border of and in intimate association with a bacterial colony. The plates were examined routinely 4 and 7 days after incubation; in 2 cases the "X" colonies were seen on the 4th day and in the third not until the 7th day, although in a repeat culture from the same tonsil many colonies appeared on the 4th day. Impression films of zones containing these colonies were unsatisfactory because the bacteria from the adjacent colonies obscured the field. Many attempts to passage the "X" colonies in series were without success. When an isolated "X" colonies in series were without success. When an isolated "X" colony was streaked on 30% ascitic fluid agar or put into broth containing 30% ascitic fluid and 0.5% glucose no growth of any kind occurred. When "X" colonies and adjacent bacterial colonies were passaged together only the bacterial colonies grew out. In 2 of the 3 cases it was possible to obtain "X" colonies several times by repeating cultures from the same tonsils which were kept in the refrigerator, but passage was invariably unsuccessful. There was thus no evidence that the "X" colonies were either a variant or a symbiont of any of the tonsillar bacteria, and their nature remains obscure. It is perhaps significant that they were not observed once among the "swab" cultures from the eyes, nose, and throat of the 100 cases studied by the same method. Swabs from the nose and tonsillar regions of the child, whose tonsils yielded the largest number of "X" colonies, were cultured 7 weeks after tonsillectomy but no "X" colonies were found. The possibility must be investigated that the "X" colonies may represent pleuropneumonia-like microörganisms which are intimately associated with certain cells and have such specific growth requirements that only one generation is possible on the 30% ascitic fluid agar, but it should be stressed that there is still no evidence that there is a human group of pleuropneumonia organisms such as has been shown to exist in cattle, sheep and goats, dogs, rats, and mice.

types of these microörganisms are injected intravenously or by certain other routes, experimental diseases are produced in mice which resemble in many respects some of the manifestations of rheumatic fever and rheumatoid arthritis in man.² Many attempts have been made, therefore, to isolate similar microörganisms from these human diseases. Failure to obtain such microörganisms from the exudates and tissues of a small number of patients with rheumatoid arthritis or rheumatic fever has already been reported¹⁻³ and the purpose of the present investigation was (a) to determine whether or not human beings may be carriers of similar or related microörganisms, and (b) to study additional material from patients with rheumatic fever or rheumatoid arthritis.

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Sabin, A. B., Science, 1938, 88, 575; ibid., 1939, 89, 228.
 Findlay, G. M., Mackenzie, R. D., and MacCallum, F. O., Brit. J. Exp. Path.,

Summary. Cultures on 30% ascitic fluid agar of material obtained by swabbing the eyes, nose, and throat of rheumatic and non-rheumatic children and a few adults failed to reveal any pleuropneumonia-like colonies. No success was encountered in additional attempts to isolate microorganisms of the pleuro-pneumonia group from the blood of children in the febrile phase of acute rheumatic fever or Still's disease, from the joint fluid during the first attack of rheumatic polyarthritis, and from rheumatic pericardial, myocardial, and valvular tissues obtained at necropsy. Cultures of 58 pairs of excised tonsils, however, yielded in 3 cases peculiar microscopic colonies ("X" colonies) which were 20 to 40 μ in size and strikingly similar to those of certain members of the pleuropneumonia group. The "X" colonies could not be passaged beyond the first generation, and their nature remains unknown.

11531 P

Pathogenic Pleuropneumonia-Like Microörganisms in Tissues of Normal Mice and Isolation of New Immunological Types.

ALBERT B. SABIN AND BARBARA JOHNSON.

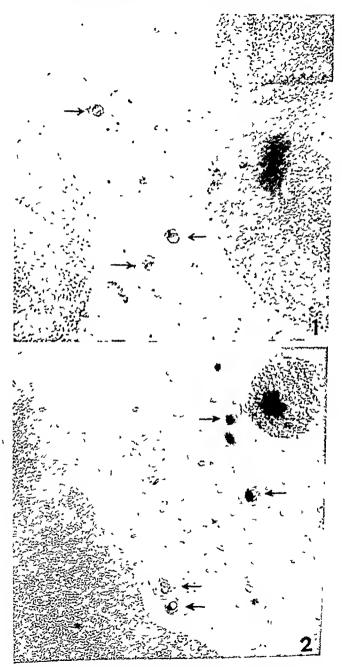
From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine.

That normal mice can be carriers of a distinct group of pathogenic pleuropneumonia-like microorganisms has already been demonstrated in an investigation of 3 different stocks of animals in New York.¹ Previous studies have established that their natural habitat was the conjunctiva and nasal mucosa,¹ although at least one strain was found in the brain of a normal mouse.² They have also been isolated from the lungs of mice which had received nasal instillation of various materials under ether anaesthesia^{1, 3} and in the brains of mice which had been used for passage of various other infectious agents.^{2, 4} Three distinct immunological types—A, B, and C—which vary in their pathogenicity and tissue affinities as well as in their antigenic make up, have now been described.

¹ Sabin, A. B., Science, 1939, 90, 18.

² Sabin, A. B., Science, 1938, 88, 575; ibid., 1939, 89, 228.

³ Sullivan, E. R., and Dienes, L., PROC. Soc. EXP. BIOL. AND MED., 1939, 41, 620.
4 Findlay, G. M., Klieneberger, E., MacCallum, F. O., and Mackenzie, R. D.,
Lancet, 1938 (Dec. 31st), 1511.



Figs 1 and 2 Growth resulting from streaking human tonsils on 30% ascitic fluid agar. Arrows point to ''X'' colonies Fig 1—X112, Fig 2—X150

few colonies were present in the cultures from the brain (there were no ordinary bacteria) and all 3 strains proved to be neurotropic exotoxin producing Type A's. Because the culture obtained from the nose of mouse 5 behaved peculiarly in tests for pathogenicity and agglutination, it was plated out and 3 different kinds of pleuro-pneumonia-like colonies were observed. Isolation and passage of single colonies revealed that the original culture was a mixture of 3 immunologically distinct types—A, D, and E. The new types D and E produce a progressive chronic arthritis but no neurotropic exotoxin and are immunologically different not only from one another but also from types A, B, and C of the mouse group and L₃ and L₄ of the rat group of pleuropneumonia-like microörganisms. Mice 11 and 12 (Table I) were sacrificed several days after the intravenous injection of bacteria, and the 3 strains of pleuropneumonia-like microörganisms which were isolated from the nose, lungs, and brain all belonged to the new type D.

Cultures from the conjunctiva and nasal mucosa of 10 old mice (6 months or older) yielded 6 strains from 4 mice, suggesting that the carrier state is probably not a transitory phenomenon. Two of these strains were typed and the one from the eye was a type A and that from the nose a type B. While type A as well as other types have been encountered in various tissues, the strains which have thus far been isolated from the eyes have all been type A. That the carrier state probably develops after birth by contact infection is suggested by a preliminary study of 5 mothers and their offspring. While 4 of the 5 mothers were carriers (nose, eyes, or both), no such microörganisms were found in 13 of their offspring at 3 days of age and were present in the nose of only 1 out of 20 at 5 days of age.

Summary. Pathogenic microörganisms of the mouse pleuro-pneumonia group in addition to being present in the conjunctiva and nasal mucosa may often be found in the brain and occasionally also in the trachea and lung of normal carrier mice. They were not found in the heart blood, liver, spleen, kidneys or in the intestinal contents. The carrier state is probably the result of contact infection and has been demonstrated as early as the 5th day of life and later than 6 months. Two new, immunologically distinct types (D and E) have been isolated; they produce arthritis but not the neurotropic exotoxin which thus far has been found to be elaborated only by the type A strains.

The purpose of the present study was to determine (a) to what extent these microorganisms were present in other tissues of carrier mice, (b) whether the carrier state persisted throughout life or was limited to a special age group, and (c) to investigate further the multiplicity of immunological and biological types that make up the mouse pleuropneumonia group. The mice used in the present studies came from an albino stock that had been inbred in Ohio for about 50 years. A preliminary investigation of the nasal mucosa and conjunctiva of 6 mice, yielded 5 new strains from 3 mice, all of which produced the neurotropic exotoxin and were immunologically type A. The distribution of the microorganisms in various tissues of carrier mice was studied in 10 animals which were 3 to 4 weeks The eyes, nose, trachea, lungs, heart, blood, liver, spleen, kidney, brain, and intestinal contents were cultured on 30% ascitic fluid agar. The intestinal contents were taken up in physiological salt solution, centrifuged at about 2000 rpm for 30 minutes, and the supernatant liquid was used for cultivation. Microörganisms of the pleuropneumonia group were obtained from 7 of the 10 mice (Table I). With the exception of the eyes and the upper respiratory tract they were isolated from the brain of 3 of these mice. Only a

TABLE I.

Pathogenie Pleuropneumonia-like Microörganisms in Various Tissues of Carrier Mice.

					Tissue:	e eultur	ed			
Mouse No.	Eyes	Nose	Trachea	Lungs	Heart blood	Liver	Spleen		Intestinal contents	
1	0	+ (A)*	0	0	0	0	0	0	0	0
2	0	` ó	0	0	0	0	0	0	0	0.
2 3	0	0	.0	0	Ō	0	Ô	0	0	+ (A)
4	0	+	0	0	0	0	0	0	0	`0
4 5	+	(A,D,E)	+	0	Uns.	Uns.	Uns.	Uns.	0	0
6	(A)	+	0 ,	0	0	0	0	0	0	+ (A)
7	1,	0	0	0	0	0	0	0	0	°o′
7 8	; (A)	0	0	ō	0	0	0	0	0	+ (A)
9	0	0	0	0	0	0	0	0	0	Ò
10.	0	Uns.	0	0	0	0	0	0	Uns.	0
11	0	+ (D)		(D)		0	0			0
12		(-)		`0'		0	0			+ (D)

^{*}Letters in parentheses refer to the immunological type of the strain that was isolated.

Uus,-culture unsatisfactory.

few colonies were present in the cultures from the brain (there were no ordinary bacteria) and all 3 strains proved to be neurotropic exotoxin producing Type A's. Because the culture obtained from the nose of mouse 5 behaved peculiarly in tests for pathogenicity and agglutination, it was plated out and 3 different kinds of pleuropneumonia-like colonies were observed. Isolation and passage of single colonies revealed that the original culture was a mixture of 3 immunologically distinct types—A, D, and E. The new types D and E produce a progressive chronic arthritis but no neurotropic exotoxin and are immunologically different not only from one another but also from types A, B, and C of the mouse group and L₃ and L₄ of the rat group of pleuropneumonia-like microörganisms. Mice 11 and 12 (Table I) were sacrificed several days after the intravenous injection of bacteria, and the 3 strains of pleuropneumonia-like microörganisms which were isolated from the nose, lungs, and brain all belonged to the new type D.

Cultures from the conjunctiva and nasal mucosa of 10 old mice (6 months or older) yielded 6 strains from 4 mice, suggesting that the carrier state is probably not a transitory phenomenon. Two of these strains were typed and the one from the eye was a type A and that from the nose a type B. While type A as well as other types have been encountered in various tissues, the strains which have thus far been isolated from the eyes have all been type A. That the carrier state probably develops after birth by contact infection is suggested by a preliminary study of 5 mothers and their offspring. While 4 of the 5 mothers were carriers (uose, eyes, or both), no such microörganisms were found in 13 of their offspring at 3 days of age and were present in the nose of only 1 out of 20 at 5 days of age.

Summary. Pathogenic microörganisms of the mouse pleuropneumonia group in addition to being present in the conjunctiva and nasal mucosa may often be found in the brain and occasionally also in the trachea and lung of normal carrier mice. They were not found in the heart blood, liver, spleen, kidneys or in the intestinal contents. The carrier state is probably the result of contact infection and has been demonstrated as early as the 5th day of life and later than 6 months. Two new, immunologically distinct types (D and E) have been isolated; they produce arthritis but not the neurotropic exotoxin which thus far has been found to be elaborated only by the type A strains.

The purpose of the present study was to determine (a) to what extent these microorganisms were present in other tissues of carrier mice, (b) whether the carrier state persisted throughout life or was limited to a special age group, and (c) to investigate further the multiplicity of immunological and biological types that make up the mouse pleuropneumonia group. The mice used in the present studies came from an albino stock that had been inbred in Ohio for about 50 years. A preliminary investigation of the nasal mucosa and conjunctiva of 6 mice, yielded 5 new strains from 3 mice, all of which produced the neurotropic exotoxin and were immunologically type A. The distribution of the microörganisms in various tissues of carrier mice was studied in 10 animals which were 3 to 4 weeks The eyes, nose, trachea, lungs, heart, blood, liver, spleen, kidney, brain, and intestinal contents were cultured on 30% ascitic fluid agar. The intestinal contents were taken up in physiological salt solution, centrifuged at about 2000 rpm for 30 minutes, and the supernatant liquid was used for cultivation. Microorganisms of the pleuropneumonia group were obtained from 7 of the 10 mice (Table I). With the exception of the eyes and the upper respiratory tract they were isolated from the brain of 3 of these mice. Only a

Pathogenic Pleuropneumonia-like Microörganisms in Various Tissues of Carrier Mice.

					Tissues	enltur	ed			
Mouse No.	Eyes	Nose	Trachea	Lungs	Heart blood	Liver	Spleen	Kidney	Intestinal contents	Brain
1	0	+ (A)*	0	0	0	0	0	0	0	0
2	0	` ó	0	0	0	0	0	0	0	0
2 3	0	0	0	0	0	0	0	Ō	0	+ (A)
4	0	+	0	0	0	0	0	0	0	`o´
4 5	+	; (A,D,E)	+	0	Uns.	Uns.	Uns.	Uns.	0	0
6	+ (A)	+ '	0	0	0	0	0	0	0	+ (A)
7	+	0	0	0	0	0	0	0	0	`0
7 8	(A)	0	0	0	0	0	0	0	0	+ (A)
9	`o´	0	0	0	0	0	0	0	0	0
10.	0	Uns.	0	0	0	0	0	0	Uns.	0
11	0	+ (D)		+ (D)		0	0			0
12		(D)		0		0	0			+ (D)

^{*}Letters in parentheses refer to the immunological type of the strain that was isolated.

Uns.—culture unsatisfactory.

were freed from the absorbent cotton with the aid of 30 cc of physiological salt solution. After horizontal centrifugation one part was injected into mice without any further treatment, another part was treated with 15% of anaesthetic ether in the same manner as the feces, and still another part was mixed with one-tenth its volume of rabbit serum and passed through a Berkefeld "V" filter which had been saturated with 10 cc of 10% rabbit serum in physiological salt solution. The other tissues were prepared as indicated in Table I. With the exception of the heart blood, subarachnoid fluid, and the centrifuged, untreated suspensions of the nasal mucosa and of the tonsillar and pharyngeal tissues, each specimen was injected into 6, two- to three-week-old white mice and one rabbit; where this number of animals does not appear in the table it means that they died within one or 2 days as a result of the inoculation.

The results shown in Table I indicate that while there was no demonstrable virus at the site of inoculation, i.e. the bitten area, there was enough present in the cervical cord and medulla, the cornu ammonis, and the olfactory bulbs to produce rabies in all the inoculated animals after a relatively short incubation period. The diagnosis of rabies was made by the demonstration of large numbers of typical Negri bodies in films of the brains of succumbing animals, by positive passage, and finally by neutralization with rabies immune serum which was kindly supplied by Dr. L. T. Webster of the Rockefeller Institute. A very small amount of virus, only enough to produce rabies in one of 6 mice, was present in the untreated, centrifuged suspensions of the nasal mucosa and of the tonsillar and pharyngeal tissues, while none was found in the Berkefeld "V" filtrates of the same preparations. Virus was also not demonstrated in the heart blood, subarachnoid fluid, saliva, and feces. affected mice and rabbits were observed for 8 weeks and were then tested for immunity by an intracerebral injection of a 10-3 dilution of the virus (derived from the cord and medulla of this case and passaged in mice) which represented approximately 10 minimal mouse cerebral lethal doses. All of 62 test mice and 15 control mice of the same age developed rabies; 5 test rabbits and one control also succumbed.

With regard to the centrifugal spread of the virus along the olfactory pathways in human rabies, it would appear therefore that after entry by way of the nerves supplying the hand the virus can spread to and be present in appreciable amounts in the olfactory bulbs; the small amount which was demonstrated in the nasal mucosa indicates how little the extracranial part of the olfactory system can

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Spread of Virus in an Unvaccinated Case of Human Rabies.

ALBERT B. SABIN AND ISAAC RUCHMAN

From the Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine.

A necropsy on an unvaccinated case of human rabies presented an opportunity to investigate several questions regarding the spread of the virus in human beings and the development of immune bodies during the course of the disease. Since rabies is noted for the centrifugal spread of the virus it was desirable to determine (particularly for correlation with poliomyelitis in man) to what extent the virus spread outwards along the olfactory pathway and whether or not it appeared in the feces in a case in which the portal of entry was known to be the hand.

The patient was a 55-year-old man who was bitten on the right hand by a dog. There was no treatment other than superficial cleansing of the wound. He died 2 months later after an illness of 2 to 3 days with a clinical diagnosis of rabies. Necropsy was limited to the head, and the following structures were investigated: (1) The skin and subcutaneous tissue at the site of the bite (which was still marked by hyperemic scar tissue) after superficial sterilization with iodine and alcohol, (2) upper cervical cord and medulla, (3) cornu ammonis, (4) olfactory bulbs, (5) nasal mucosa (mostly olfactory) removed after an intracranial exposure of the roof of the nasal cavity, (6) tonsillar and pharyngeal tissues, (7) saliva obtained by swabbing out the mouth and throat with sterile absorbent cotton, (8) feces removed from the rectum by spatula, (9) subarachnoid fluid over the exposed cerebral cortex removed with a needle and syringe, and (10) heart blood. The feces was prepared for inoculation in two different ways: (a) one 2 g sample was shaken with glass beads in 10 cc of broth and after horizontal centrifugation at about 2000 rpm for 15 minutes the supernatant liquid was passed through a Berkefeld "V" filter which had previously been saturated with 20 cc of broth; (b) a 2.5 g sample was shaken in 15 cc of distilled water and after centrifugation as above, the supernatant liquid was mixed with 2 cc of anaesthetic ether and thoroughly shaken for 10 minutes; after 5 hours in the refrigerator at approximately 5°C the mixture was again centrifuged and the very opalescent fluid portion removed. Preparation (a) was bacteria-free while preparation (b) was not. The saliva and mucus

were freed from the absorbent cotton with the aid of 30 cc of physiological salt solution. After horizontal centrifugation one part was injected into mice without any further treatment, another part was treated with 15% of anaesthetic ether in the same manner as the feces, and still another part was mixed with one-tenth its volume of rabbit serum and passed through a Berkefeld "V" filter which had been saturated with 10 cc of 10% rabbit serum in physiological salt solution. The other tissues were prepared as indicated in Table I. With the exception of the heart blood, subarachnoid fluid, and the centrifuged, untreated suspensions of the nasal mucosa and of the tonsillar and pharyngeal tissues, each specimen was injected into 6, two- to three-week-old white mice and one rabbit; where this number of animals does not appear in the table it means that they died within one or 2 days as a result of the inoculation.

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+						
+						
21,0,0,0,0,0 0,0,0,0,0	0	(3 bact.); 1d%,0,0 0,0,0,0,0,0	0	0,0,0,0,0,0	0,0,0,0,0	0,0,0,0,(2 bact.)
6 mico 6 ,,	1 rabbit	6 mieo 6 ,,	1 rabbit	6 mice	(1 9	6 ,,
i.eer.	i.cor. massoter	i.eer.	i.eor.	i.eer. i.a.	i.eer.	i.cer.
.0. 80.	0.1 0.12 73	.03 .03		1.03	.03	.03
Centrif. susp. Berkefeld "V" filt.		Centrif. susp. Berkefold (TV") filt.		Ether-treated	Berkefold "V" filt.	Ether-treated
Tonsillar and pharyngeal tissues	10% suspension in 10% rabb. ser.	Saliva			Foces	

*Numerals refor to day on which signs of nervous system involvoment were first observed in mice. One of the carliest signs (which we have never observed in mico injected with other viruses) consisted of a momentary fluring of the cars when the mico were picked up with a forceps and dropped back into the cage.

Other abbreviations: i.cer. = intracrebral; i.a. = intraibdominal; 3 bact. = 3 mico died of bacterial infection; 1d? = mouse died ou 19th day but because it had been chewed by its mates neither passage nor examination for Negri bodies or bacterial infection ICNS 3, D9-Signs of nervous system involvement 8th day and dead 9th day. was possible.

TABLE I. Distribution of Virus in an Unvaccinated Case of Human Rabics.

	Established of virus in an Unvaccinated Case of Huunan Rabies,	s in an	Unvacemater	l Casc of Human R	abics,		
Material tested	Preparation for inoculation	Amt	Site	Animals inoculated	Result	Negri	Negri hodies Passage
Skin lesion (site of bito)	Centrifugod suspension	. v.	i. cer.	6 mice 1 rabbit	0,0,0,0,0,0		Specie
		ဗ္	massctor		'n		
lleart blood	•	.03	i.cer.	6 miec	0.0.0.0.0		
Subarachnoid fluid over cortex	•	.03	:	23 33	0.0.0.0.0		
Upper cervical cord and medulla	10% susp.	.03	: :	11 11	8*,10,10,11,11,11	+	+
			i.cor. i.a.	1 rabbit	CNS 8, D0+	+	- +
Cornu ammonis		.03	i.cor.	4 mico	13,13,14,14	+	+
Offactory bulbs	Ground in 1 cc salinc	.03	i.ccr.	6 '' 1 rabbit	12,12,13,13,13,14 CNS 10, 13,14	+-	+
Olfactory mucosa 10% suspension in 10% rabb. ser.	Centrif. susp. Berkefeld ''Ų''' filtrato	.03 .03		6 micc 6 ",	14,0,0,0,0,0 0,0,0,0,0	+ +	+ +
		rċ rċ	i.cor. massoter	1 rabbit	0		

VIRUS SPREAD IN H	UMAN KADIL
(3 baet.); 1d ⁴ ,0,0 0,0,0,0,0,0 0 0,0,0,0,0,0 0,0,0,0,0,0 0,0,0,0,0,0 0,0,0,0,	Foces Foces Flower to the constant of the co
i.cor. 6 mico i.cor. 1 rabbit i.cor. 6 "" massetor 6 "" i.a. 1 rabbit i.cor. 6 "" i.cor. 6 "" i.cor. i.a. 1 rabbit i.cor. i.a. 6 mico i.a. 6 "" i.cor. i.a. 6 "" i.cor. i.a. 6 "" i.cor. i.a. 6 "" i.cor. i.a. 6 ""	nvolvemoul were first observed insisted of a monontary flar and dend oth day: Ind dend oth day: Indoninal; 3 back. = 3 mice indicate passago nor examina
1.0 1.0 1.0 1.0 1.0 1.0 1.0 1.0	ystem in uses) co 3th day c intraü s mates
Centrif. susp. filt. Berkefeld '(V')' filt. Berkefeld '(V')' filt. Ether-treated Borkefeld '(V')' filt.	Ether-treated on which signs of nervous injected with other vir. pred back into the cage. pred back into the cage. intravous system involvement i.a. intravorebral; i.a. intravorebral; i.a. integer it had been chewed by it
Tonsillar and pharyngeal tissucs 10% rabb. ser. Saliva	Foccs "Numorals refer to day we have never observed in we have never observed in we have never observed in the never never of the never of the never abbreviations: i died on 19th day but becau was possible.

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	The state of the s	o til ali	Out account to	r manual to aspect	ta ores.		
Material tested	Preparation for inoculation	Amt	Site	Animals inoculated	Result	Negri bodies	Passage
Skin lesion (site of bito)	Centrifuged suspension	.03	i. cer.	6 mico 1 rabbit	0,0,0,0,0,0		
Heart blood		.03	i.cer.	6 mieo	0.0,0,0,0		
Subaraehnoid fluid over cortex	• • • • • • • • • • • • • • • • • • • •	.03	•	33 33	0.0.0.0.0		
Upper cervical cord and medulla	10% susp.	.03	÷ .	"	8*,10,10,11,11	+	+
		20.03	i.cor.	1 rabbit	CNS 8, D9	+	+
Cornu ammonis		.03	i.cer.	4 mice	13,13,14,14	+	+
Olfactory bulbs	Ground in 1 cc saline	.03	i.cer.	6 ", 1 rabbit	19,12,12,13,13,14 CNS 10. D11	++	+-
Olfactory nucosa 10% suspension in 10% rabb. ser.	Centrif. susp. Berkefeld '(V')'	.03 1.0	;;;	6 mice 6 ,,	14,0,0,0,0,0 0,0,0,0,0,0	- +	+ +
		ကဲ့ ကဲ့	i.cer. masseter	1 rabbit	0		

bodies would be present in an individual who died 2 months after the introduction of rabies virus into the body. The question was of some interest first, because it is known that neutralizing antibodies appear in animals and human beings within 2 to 3 weeks after the injection of effective rabies vaccines, and second, because in diseases like equine encephalomyelitis and vellow fever in which the viruses are viscerotropic as well as neurotropic, neutralizing antibodies are often found in animals and human beings succumbing to the infection. The patient's serum obtained post-mortem was tested against the virus which was isolated from his spinal cord and medulla and passaged in mice 8 times; an immune serum from mice inoculated with a fixed strain of rabies virus (supplied by Dr. Webster) was tested simultaneously. Mixtures of equal parts of the test sera and various dilutions of the virus were injected intracerebrally in mice. The results, shown in Table II, indicate that the patient's serum had no neutralizing antibodies against the virus, while the mouse immune serum exhibited definite protection.

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Summary. In a 55-year-old man who died of rabies two months after a bite on the hand, virus was not demonstrated at the site of the bite but was present in appreciable amounts in the cervical cord and medulla, the cornu ammonis, and the olfactory bulbs; only a trace was found in the nasal mucosa and the tonsillar and pharyngeal tissue, and none was found in the feces, saliva, subarachnoid fluid, and heart blood. The patient's serum obtained post-mortem had no neutralizing antibodies for the virus.

be affected. While the failure to demonstrate virus in the saliva and feces may partly be due to inadequate methods, it may also be that there was insufficient centrifugal spread of the virus. The present status of the presence of virus in the salivary glands or saliva in human rabies is rather indefinite. Thus, Williams, stated that "glands from human beings are seldom infective for test animals," and Leach² recently reported isolation of the virus from the salivary glands of only one of 3 human cases. Kraus, Gerlach, and Schweinburg³ pointed out the discordance in the results and opinions concerning infectivity of saliva, and added that no case of rabies has been known to be produced by the bite of a human being. Palawandow and Serebrennaja reported that they produced rabies in wandow and Screptennaja reported that they produced rapies in guinea pigs [the evidence is not unequivocal, however] by intramuscular injection of saliva from a 12-year-old girl with rabies, and according to Pawans the above authors obtained similar results with saliva from 5 rabid persons. Pawans tested the saliva of 6 persons with signs of paralytic rabies (Trinidad) by rubbing swabs moistened with saliva into the scarified abdominal wall of 7 rabbits, all of which became paralyzed and exhibited Negri bodies; by a similar method he demonstrated the virus in the saliva of bovines,

The other question to be investigated was whether or not immune horses, and vampire bats.

TABLE II.

TABLE II.

Tables Virus in Patient's Post-mortem

Against Rabies Virus in Patient's Post-mortem

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The other question	TABLE	11. Virus in P	111022	
The our	aginst I	Rables		
Antil	odies Ag Serun	1.		
Neutralizing Mar		Posult with		
Test for New		Result	Mouse immuue	
		Patient's	serum	
	المسسسم	serum		
	Breth		8,10,10,0	
Dilution	control	4,5,5,6	19.0.00	
of virus		5,7,7,8	10 0.0,0	
	5,5,5,6*	6,7,7,15	0,0,0,0	
10-2	C 15 1.0	7,7,0,0	n.t.	
10-3	7,8,10,0	7,1,0,	* 07	ebrally
70-4	0,0,0,0	11	i jected intracer	vement
10 -5	0,0,0,0	inture was	injects of invol	, 0-
10-6	of	each mixture on wh	niell signed.	
10-4	ous dilutions of	r to the day n.t. =	= not test	
10-2 10-3 10-4 10-5 10-6	ie numerals fero	t observed.		r - 1,11501

^{*0.03} ee of the various dilutions of each mixture was injected intracerebrally *0.03 ee of the various anutions of each mixture was injected intracerebrany into each of 4 nice. The numerals refer to the day on which signs of involvement into each of 4 nice. The numerals refer to the day on which signs of involvement into each of 4 nice. The numerals refer to the day on which signs of involvement into each of 4 nice. into each or 4 mise. The numerals refer to the day on which signs of of the nervous system (rables) were first observed. n.t. = not tested.

² Leach, C. N., personal communication; see also Leach, C. N., and Johnson, Leaen, C. N., personal communication; see also Leaen, C. N., and Jonuson, H. N., Abstracts of communications, Third International Congress for Micro-1 Williams, A. W., Abt's Pediatrics, 1925, 6, 251.

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3 Kraus, R., Gerlaeh, F., and Schweinburg, Lyssa bei Mensch und Tier, Vienna,

^{20,} pp. 00, 120-101. 4 Palawandow, H., and Serebrennaja, A. I., Z. f. Immunitätsforsch., 1930, 1926, pp. 80, 128-131.

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TABLE II.

Test for Neutralizing Antibodics Against Rabies Virus in Patient's Post-mortem Serum.

		Result with	
Dilution of virus	Breth control	Patient's serum	Mouse immune serum
10-2	5,5,5,6*	4,5,5,6	8,10,10,0
10-3	6,6,7,8	5,7,7,8	12,0,0,0
10-4	7,8,10,0	6,7,7,15	10,0,0,0
10-5	0,0,0,6	7,7,0,0	0,0,0,0
10-6	0,0,0,0	n.t.*	n.t.

*0.03 cc of the various dilutions of each mixture was injected intracerebrally into each of 4 mice. The numerals refer to the day on which signs of involvement of the nervous system (rables) were first observed. n.t. = not tested.

¹ Williams, A. W., Abt's Pediatrics, 1925, 6, 251.

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bodies would be present in an individual who died 2 months after the The question was of introduction of rabies virus into the body. some interest first, because it is known that neutralizing antibodies appear in animals and human beings within 2 to 3 weeks after the injection of effective rabies vaccines, and second, because in diseases like equine encephalomyelitis and yellow fever in which the viruses are viscerotropic as well as neurotropic, neutralizing antibodies are often found in animals and human beings succumbing to the infection. The patient's serum obtained post-mortem was tested against the virus which was isolated from his spinal cord and medulla and passaged in mice 8 times; an immune serum from mice inoculated with a fixed strain of rabies virus (supplied by Dr. Webster) was tested simultaneously. Mixtures of equal parts of the test sera and various dilutions of the virus were injected intracerebrally in mice. The results, shown in Table II, indicate that the patient's serum had no neutralizing antibodies against the virus, while the mouse immune serum exhibited definite protection.

The results obtained in the present investigation throw some additional light on the behavior of rabies virus in man, but it will, of course, be necessary to carry out similar studies in other unvaccinated cases to establish whether or not the present findings are the exception or the rule.

Summary. In a 55-year-old man who died of rabies two months after a bite on the hand, virus was not demonstrated at the site of the bite but was present in appreciable amounts in the cervical cord and medulla, the cornu ammonis, and the olfactory bulbs; only a trace was found in the nasal mucosa and the tonsillar and pharyngeal tissue, and none was found in the feces, saliva, subarachnoid fluid, and heart blood. The patient's serum obtained post-mortem had no neutralizing antibodies for the virus.

11533 P

Plasma Protein Shifts During Diuresis.

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Galveston,

In a previous communication¹ changes were described in the plasma volume after the injection of salyrgan, aminophyllin, and digoxin. This report deals with the plasma protein changes under these conditions. Total proteins were determined by the Kjeldahl technic; albumin by the method of Campbell and Hanna; plasma volumes by the method of Gregerson, Gibson and Stead³ as modified by Gibson and Evelyn.⁴

After the injection of salyrgan, as the plasma volume drops there is a concentration of proteins, with a rise in the percentage of total protein and a relatively greater increase in albumin, leading to an increase in the albumin: globulin ratio. When the plasma volume is markedly lowered following diuresis the percentage of plasma protein increases persist, but the total amount of circulating protein is conspicuously lowered.

Paralleling the increase in plasma volume after aminophyllin there is a fall in percentage of plasma protein, but an absolute increase in the amount of total circulating protein. The latter is largely accounted for by an increase in circulating albumin, resulting in an increase in the albumin globulin ratio. During the subsequent drop, usually marked, in blood volume there is a definite rise in percentage of total protein and albumin, though the amount of each in the total circulating plasma shows a striking drop.

When digoxin was used, the shifts in plasma protein were less regular, but in general as the plasma volume dropped there was a rise in the percentage of total protein and albumin, with little early change in the total amounts of each in the circulating plasma. When however the decrease in the plasma volume became marked, though the percentage values were maintained there was a large loss of protein from the circulating plasma.

¹ Calvin, D. B., Decherd, George, and Herrmann, George, Proc. Soc. Exp. Biol. AND Med., 1946, 44, 529.

² Campbell, W. R., and Hanna, M. I., J. Biol. Chem., 1937, 119, 15.

³ Gregerson, M. I., Gibson, J. G., and Stead, E. A., Am. J. Physiol., 1935, 113, 54.

⁴ Gibson, J. G., and Evelyn, K., J. Clin. Invest., 1938, 17, 153.

TABLE I.

		Dlasma	Total protein		Albumin		
Drug	Hr after injection	Plasma volume, ec	g%	g total circulating	g%	g total circulating	A/G ratio
Salyrgan	0	5140	5.74	295	3.13	162	1.20
MV	1/2	5110	5.91	302	3.26	166	1.23
		4620	6.02	279	3.45	159	1.34
	1 2 5	4620	6.11	283	3.46	161	1.31
	5	3280	6.20	205	3.63	119	1.42
Aminophyllin	ı 0	6120	6.10	373	3.38	207	1.24
AO T	1/2	6488	6.05	393	3.34	223	1.22
		6383	5.94	380	3.32	212	1.27
	1 2 3 5 7	6500	hemol	ysis			
	3	6697	6.01	402	3.39	227	1.30
	5	7202	5.87	423	3.43	242	1.41
	7	6278	6.25	393	3.63	228	1.39
	9	4913	6.39	314	3.62	178	1.40
	11	4072	6.44	263	3.81	156	1.45
Digoxin	0	3890	5.76	224	3.24	126	1.29
НŘ	1	4130	5.62	232	3.24	134	1.36
	2	3640	6.00	218	3.50	127	1.40
	3	3682	5.87	216	3.36	124	1.34
	1 2 3 5 7 9	3800	5.63	214	3.22	. 122	1.34
	7	3790	hemol	ysis			
		3060	5.94	182	3.63	111	1.57
	11	2505	6.06	152	3.65	92	1.51

Whenever there is an increase in the plasma volume after the injection of aminophyllin, there is noted an increase in the total circulating protein, chiefly albumin, similar to the changes observed in experimental hydremia in the dog. On the other hand, with each type of diuretic a drop in plasma volume is associated with moderate increase in the percentage of total plasma protein due largely to an increase in plasma albumin. The marked drop in the total circulating proteins under these circumstances seems explicable only on the assumption of plasma protein storage in the tissues.

⁵ Calvin, D. B., Proc. Am. Physiol. Soc., in press.

11534

Administration of Ascorbic Acid to an Alkaptonuric Patient.*

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Recently it has been reported from the senior author's laboratory that artificial alkaptonuria may be readily produced in the guinea pig by the supplementation of a vitamin C deficient diet with extra tyrosine.1 With the subsequent administration of the vitamin the homogentisic acid in the urine was promptly reduced in amount and within 48 to 72 hr was completely absent. These findings suggested that the relation of ascorbic acid to the excretion of homogentisic acid by the alkaptonuric patient should be investigated. However, in the course of the experiments with the guinea pigs, the results of two such investigations appeared. These reports by Monsonyi2 and by Diaz, Mendoza and Rodriguez³ indicate that ascorbic acid is without effect, but since, in our own more recent studies* it has been apparent that in the guinea pig the effectiveness of the dose of ascorbic acid is dependent upon the state of vitamin saturation in the tissues, it was considered imperative to investigate the effect of doses of the vitamin greatly in excess of the normal human requirement, and also of the relatively small amounts used by the above investigators.

Experimental. The individual who served as a subject for this study is an essentially normal 65-year-old white male who exhibited at the time of these experiments alkaptonuria and ochronosis—a deposition of pigment mainly in cartilage which is characteristic of these individuals in later years. Since the patient had previously been on an experiment in which he consumed a relatively high intake of protein, he was continued on this level, the diet being a mixed diet with considerable variety from day to day but so planned that it

^{*} Aided by a grant from the Committee on Scientific Research of the American Medical Association.

¹ Sealock, R. R., and Silberstein, H. E., Science, 1939, 90, 517.

² Monsonyi, L., Presse Med., 1939, 47, 708.

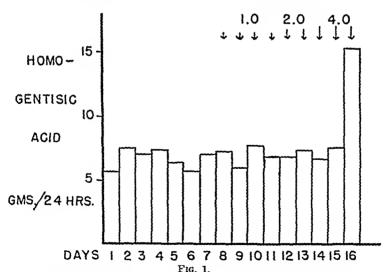
³ Díaz, C. J., Mendoza, H. C., and Rodríquez, J. S., Klin. Wchnschr., 1939, 18, 965.

⁴ Sealock, R. R., Perkinson, J. D., and Silberstein, H. E., in press.

furnished approximately 130 g of protein per day. It included throughout the experiment 40 to 50 mg of vitamin C daily, an amount comparable to the previous daily intake of the patient. The urine was collected in 24 hr samples in bottles containing sufficient hydrochloric acid to make the sample slightly acid to congo red. The homogentisic acid was determined by the iodine-sodium thiosulfate titration procedure of Metz⁵ as modified by Lieb and Lanyar. The ascorbic acid determinations were made in the usual fashion by titration of the fresh sample with standardized 2,6-dichloro-benzenone-indophenol. Since homogentisic acid also reduces the dye under the conditions of the titrations the values were corrected for the amount of the hydroquinone derivative.

Results. Following a control period vitamin C₁ was given orally at the comparatively high level of 1.0 g per day. As shown in Fig. 1, this amount for 4 days proved to be without effect on the excretion of the metabolite, as was the further administration on the 12th and •

ALKAPTONURIA ADMINISTRATION OF VITAMIN C



Mr. G—130 g protein per day. Beginning of last day also received 10 g of l-tyrosine. Ascorbic acid, g. —

⁵ Metz, E., Biochem. Z., 1927, 190, 261.

⁶ Lieb, H., and Lanyar, F., Z. physiol. Chem., 1929, 181, 189.

[†] The vitamin used in this study was very kindly supplied by Merek and Company, Incorporated, and Chas. Pfizer and Company, Incorporated.

13th days of 2.0 g in 0.5 g portions. For the following 3 days the ascorbic acid was again doubled, 2 of the 4 g being given by intravenous injections of 1 g each. That a high level of tissue saturation had been attained was indicated by the urinary excretion of 1.08, 1.62, 1.82 and 2.67 g of ascorbic acid on the 12th to the 15th days and a blood ascorbic acid of 2.89 mg per 100 cc. In view of the failure of even 4.0 g of the vitamin to effect the homogentisic acid output, the possibility yet remained of establishing a relationship between the two by flooding the metabolic processes with an extra dose of the precursor. In order to test this possibility 10 g of 1-tyrosine were given at the beginning of the 16th day and subsequently four 1 g doses of ascorbic acid. The experiment resulted negatively, for in the 24 hr urine sample there were present 15.2 g of homogentisic acid, which when compared to the previous average represents a recovery of 88% of the theoretical.

The administration of the vitamin was not entirely without advantage, albeit the advantage was unrelated to the metabolism of the alkaptone substance. Whereas the urine samples of the control period showed the usual tendency to darken on standing, with excretion of extra ascorbic acid there was no longer any discoloration even after several days, a finding which again illustrates the well known protective action of this substance against the oxidation of ortho- and para-diphenolic compounds by atmospheric oxygen.

It should be pointed out that the large doses of ascorbic acid were without visible effect on the ochronosis exhibited by the individual. However, these results do not preclude the possibility of a continued high intake of the vitamin in early life preventing the deposition of melanotic pigment in later years. That such a possibility is of some importance is evident from the fact that perhaps the only unpleasant feature of the condition is the appearance of melanin pigment in the cartilage of the ears and nose as discussed by Garrod.

The ineffectiveness of ascorbic acid on the abnormal metabolism of the alkaptonuric individual indicates a real difference between this type of experiment and the one with the guinea pigs^{1,4} or in other words between hereditary and experimental alkaptonuria. When one recalls that the majority of metabolic reactions are chain reactions proceeding under the influence of many different factors and enzyme systems, the above difference is not surprising. In the guinea pig the missing factor is ascorbic acid while in the alkap-

⁷ Garrod, A. E., Inborn Errors of Metabolism, Oxford University Press, 1923, p. 58.

tonuric patient it is not the vitamin but some other factor as yet

Summary. The administration of from 1 to 4 g of ascorbic acid per day has been found to be without effect on the excretion of homogentisic acid by an alkaptonuric individual excreting an average of 7 g of the metabolite daily. The ineffectiveness of this excessive amount of vitamin is a finding which is in agreement with results with smaller doses obtained by other investigators.

The authors are indebted to Professor Vincent du Vigneaud for placing at their disposal laboratory facilities in the Department of Biochemistry of the Cornell University Medical College for the chemical work in connection with this experiment, and to Dr. Elaine P. Ralli of the Department of Medicine of New York University for the determination of the blood ascorbic acid.

11535 P

Relation of Pantothenic Acid to Dermatitis of the Rat.*

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Richardson and Hogan¹ demonstrated that rat dermatitis (acrodynia) is prevented or cured by an aqueous extract of yeast or rice bran, and by certain oils. The activity of the aqueous extracts was ascribed to the presence of a vitamin, presumably the one later designated as vitamin B₆. Since this communication is not immediately concerned with oils our observations, and those of other workers,² on their protective action will not be described. Birch³ reported that two factors are required to prevent or cure acrodynia. One is vitamin B₆, the other is one of the essential unsaturated fatty acids. György and Eckhardt⁴ obtained complete protection with a

^{*} Contribution from the Department of Agricultural Chemistry, Missouri Agricultural Experiment Station Journal Series No. 680.

¹ Richardson, L. R., and Hogan, A. G., Mo. Agr. Exp. Sta. Res. Bul., 1936, No. 241.

² Salmon, W. D., and Goodman, J. G., J. Nutr., 1937, 13, 477; Schneider, H., Steenbock, H., and Platz, Blanche R., J. Biol. Chem., 1940, 132, 539.

³ Birch, T. W., J. Biol. Chem., 1938, 124, 775.

⁴ György, P., and Eckhardt, R. E., Nature, 1939, 144, 512.

combination of vitamin B_6 and various filtrates after fuller's earth adsorption. Under our experimental conditions both pantothenic acid and vitamin B_6 are required to cure this type of rat dermatitis, but additional time will be required to determine whether the cure is permanent.

The rats receive a ration of casein 20, sucrose 71, cellulose 3, cod liver oil 2, and salts 4, supplemented with 0.2 mg thiamin and 0.4 mg riboflavin per 100 g of food. After mild dermatitis developed vitamin B_0 was supplied to Group I, pantothenic acid to Group II, and both were supplied to Group III. Group IV contained three rats that had become moribund on vitamin B_0 and were then rescued

TABLE I. A Combination of Pantothenic Acid and Vitamin \mathbf{B}_6 Cures Rat Dermatitis.

		Avg Exp.		g wt	_
				l. Fina	1,
Supplement	$_{ m \mu g}$	days	g	g	Remarks
B_{0}	10	20.5	47	54	All improved, 4 had re- currence, died. 1 nor-
$\mathbf{B_{6}}$	20	13	39	42	mal last observation. All improved, 3 had re- currence, 2 died. 1 nor-
$\mathbf{B}_{\mathbf{G}}$	30	18	40	37	mal last observation. Improved slightly, died.
Sodium pantothenate*	70	9.3	40	34	2 healed. I died though norm. in appearance, I recurrence. I improved but died before it was
Calcium pantothenate†	70	12	39	45	normal. 4 died without improving. All improved but not nor- mal. 2 had recurrence at last observation.
					n
$B_6 10 + Na$	70	30	50	116	Dermatitis disappeared, norm.
B ₆ 20 + Ca pantothenate	70	12	44	72	Dermatitis disappeared, norm.
Na pantothenate	70	16	52	90	Dermatitis disappeared,
,, ,,	70	15	60	67	Dermatitis disappeared, but infection in both eyes, died.
	B ₀ B ₆ Sodium pantothenate* Calcium pantothenatet B ₆ 10 + Na pantothenate B ₆ 20 + Ca pantothenate	B ₀ 10 B ₆ 20 B ₆ 30 Sodium	Supplement μg Exp. period, days B ₀ 10 20.5 B ₀ 20 13 B ₀ 30 18 Sodium pantothenate* 70 9.3 Calcium pantothenate pantothenate 70 12 B ₆ 10 + Na pantothenate pantothenate pantothenate 70 30 B ₆ 20 + Ca pantothenate 70 12 Na pantothenate 70 16	Supplement μg Exp. period, Initia days Initia days Initia days g B ₀ 10 20.5 47 B ₀ 20 13 39 B ₀ 30 18 40 Sodium pantothenate* 70 9.3 40 Calcium pantothenate* 70 12 39 B ₀ 10 + Na pantothenate 70 30 50 B ₀ 20 + Ca pantothenate 70 12 44 Na pantothenate 70 16 52	Exp. period, Initial, Fina days g g B ₀ 10 20.5 47 54 B ₆ 20 13 39 42 B ₆ 30 18 40 37 Sodium pantothenate* 70 9.3 40 34 Calcium pantothenate 70 12 39 45 B ₆ 10 + Na pantothenate 70 12 44 72 Na pantothenate 70 12 44 72

^{*}We are greatly indebted to Dr. T. H. Jukes who supplied us with this preparation. The amount is calculated from his assay in chick units.

†We are greatly indebted to Merek and Co., Rahway, New Jersey, who supplied us with this preparation.

with sodium pantothenate. Our observations are summarized in Table I.

All rats that received vitamin B₆ improved, but 9 of them had a recurrence within an average of 15 days. Of the other 2, one has been receiving vitamin B₆ for 12, the other for 30 days. Of the rats that received a pantothenic acid salt, 2 were cured. One of these was normal at death and the other had a mild recurrence of the dermatitis at the last observation. Some of the others improved but as yet none has made a complete recovery. When both vitamins were supplied simultaneously the animals recovered within a week and made considerable gains in weight. Three other rats, which failed to recover on vitamin B₆ alone and were on the point of death, responded in a similar manner when a pantothenic acid salt was also supplied. One animal died after the dermatitis had disappeared, from a secondary infection in both eyes.

The eyes of the animals which do not respond to vitamin B_{σ} alone are affected more severely than those of the animals which do not respond to pantothenic acid salts alone. The lids adhere and are soon covered by a large scab. If pantothenic acid is supplied in addition to vitamin B_{σ} at this stage the scab falls off, leaving a spectacled appearance which disappears without any additional treatment as the hair grows back in the denuded areas. Up to the present the other lesions characteristic of this type of dermatitis, if either vitamin B_{σ} or a pantothenate is supplied singly, are indistinguishable.

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Potencies of Vitamin K, and of 2-Methyl-1, 4-Naphthoquinone.

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In a previous investigation we have confirmed the report of the marked antihemorrhagic activity of 2-methyl-1,4-naphthoquinone. A careful comparison of the potencies of this compound and of pure vitamin K₁ by our 18-hour procedure showed that the latter is

^{*} We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

approximately one-half as active as the former. In view of this observation and the report that by the 6-hour procedure vitamin K_1 is only 1/30 as potent as 2-methyl-1,4-naphthoquinone, it seems desirable to publish the results that we have obtained in a comparison of the potencies of the two compounds by the 6-hour observation period.

Experimental. In our experiments, we have used for the evalua-

TABLE I. Bioassay of Vitamin K₁ and 2-Methyl-1,4-Naphthoquinone.

					Response	
			Chicks	Cho	otting time	Prothrombin
Exp.	.	Dosage	used,*	<10 min	Mean; S.E.	time, Mean; S.E.
No.	Compound	$\mu \mathbf{g}$	No.	%	min	sec
1	Vitamin K1 f	0.50	9	11	71.0 ± 24.2	
	_	1.00	10	30	23.0 ± 4.6	
	2-Methyl‡	0.50	14	50	13.1 ± 2.6	
	Controls	none	8	0	>180.	
2	Vitamin K1	2.00	10	90	7.3 ± 1.5	
_		4.00	9	100	4.5 ± 0.46	
	2-Methyl	0.50	9	67	17.2 ± 4.5	
		1.00	ğ	100	5.3 ± 0.90	
	Controls	none	9	0	>180.	
3	Vitamin Ki	1.50	14	86	6.0 ± 1.8	
•	2-Methyl	0.50	10	50	12.1 ± 2.1	
	Controls	none	10	0	110.	
4	Vitamín K1	2.00	19	100	4.5 ± 0.46	
	2-Metbyl	1.00	20	100	5.2 ± 0.83	
	Controls	none	10	0	100.	
5	Vitamin K1	1.00	20	75	8.0 ± 0.8	46.6 ± 2.4
•	2-Methyl	0.25	15	40	25.6 ± 8.2	76.6 ± 4.3
		0.50	30	70	12.0 ± 2.7	47.0 ± 3.0
	Controls	none	10	0	325.	93.3
6	Vitamin K1	2.00	15	100	5.8 ± 0.5	28.7 ± 1.2
-	2-Methyl	1.00	15	100	5.5 ± 0.4	33.7 ± 0.8
	Controls	none	5	0	361.	99.0

^{*}Chicks used in Exp. 4 were 15 days of age; all others 21.

Natural vitamin K1 was used in these experiments.

tUsed in this table as an abbreviation for 2-methyl-1,4-naphthoquinone.

¹ Thayer, S. A., Binkley, S. B., MacCorquodale, D. W., Doisy, E. A., Emmett, A. D., Brown, R. A., and Bird, O. D., J. Am. Chem. Soc., 1939, 61, 2563.

² Ansbacher, S., and Fernholz, E., J. Am. Chem. Soc., 1939, 61, 1924.

³ Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., Proc. Soc. Exp. Biol. and Med., 1939, 41, 194.

⁴ Ansbacher, S., Fernholz, E., and MacPhillany, H. B., Proc. Soc. Exp. Biol. AND Med., 1939, 42, 655; Ansbacher, S., Fernholz, E., and Dolliver, M. A., Proc. Soc. Exp. Biol. and Med., 1940, 43, 652.

tion of the response of the chicks: (1) the percentage of chicks showing a clotting time of less than 10 minutes;³ (2) the mean clotting time; (3) the mean prothrombin time.⁵ Following Ansbacher's suggestion, a solution of the compound in cod liver oil was administered and the blood drawn 6 hours later for the evaluation of the reaction. Each assay included the response of the same lot of deficient chicks to the administration of one or 2 dosages of each compound and the mean clotting time of a control group. The data are summarized in the table.

In Experiments 1, 2, 3 and 4 the volume of cod liver oil used for administration of the compounds was 0.10 cc; in Experiments 5 and 6 only 0.05 cc was used. From other reports and the data of this paper it appears likely that in experiments in which the response is restricted to a period of 6 hours or less the volume of oil used may play a rôle in the absorption of vitamin K₁ and therefore in the apparent potency. We believe that the first 4 experiments of the table (0.10 cc oil used) indicate clearly that vitamin K₁ is approximately one-third as potent as 2-methyl-1.4-naphthoquinone, whereas the more complete data of Experiments 5 and 6 in which only 0.05 cc of solvent was used show that the vitamin is at least one-half as active.

Since the relative inactivity of vitamin K₂ with respect to 2-methyl-1,4-naphthoquinone and a purified extract of alfalfa is one of the important points in the claim that an antihemorrhagic compound more active than vitamin K₁ is present in alfalfa the discrepancy between Ansbacher's data and our observations should be examined. In his recent report Ansbacher4 gives for the minimal effective dose of 2-methyl-1,4-naphthoquinone 0.5 µg and for vitamin K1 15 μg. The table in this paper shows that in the 4 experiments in which 0.50 µg of 2-methyl-1,4-naphthoquinone was administered the percentages of the groups showing clotting times of less than 10 minutes were 50, 67, 50 and 90; with 1.0 µg the response was always 100%. Consequently, it appears that the agreement with Ansbacher's data in the case of this compound is entirely satisfactory and that the discrepancy is due to the difference in the results obtained with vitamin K1. In our experiments the response to quantities of from 1 to 2 µg ranged from 30 to 100%. The highest ratio of potencies of the two compounds (Exp. 2) was about 3:1; the lowest (Exp. 5) less than 2:1.

Since the purity of the vitamin K1 used in these experiments is an

⁵ Almquist, H. J., and Klose, A. A., Brochem. J., 1939, 33, 1055.

important point, we wish to state that it was prepared by hydrolysis of the pure diacetyl dihydrovitamin K_1 . The vitamin K_1 was purified by recrystallization at -70°C.

Analysis: Found C 82.39%; H 10.37% Calculated C 82.62%; H 10.26% $E_{1 \text{ cm}}^{1\%}$ of a hexane solution at λ 249 m μ = 448

11537

Effect of Leukotaxine on Cellular Permeability and on Cleavage Development.

VALY MENKIN

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Previous studies have demonstrated the presence of a nitrogenous substance in inflammatory exudates capable per se of increasing capillary permeability and of inducing rapid diapedesis of polymorphonuclear leukocytes. This substance has been named leukotaxine. 1-3 Its isolation has offered a reasonable explanation for two of the basic mechanisms in the development of the inflammatory reaction. Furthermore, this substance has been shown to possess none of the manifest physiological properties of histamine, thus rendering it difficult to accept the view that the latter plays a primary rôle in increasing capillary permeability at the site of injury. 4-3 Kaiser has recently reached in regard to histamine an essentially similar conclusion, i. e., at least as far as a long-standing inflammatory reaction is concerned. 6

The present series of experiments have been undertaken in an endeavor to determine whether leukotaxine exerts any direct effect

^{*} Aided by grants from the Milton Fund of Harvard University, from the International Cancer Foundation, and the Dazian Fund for Medical Research.

¹ Menkin, V., J. Exp. Med., 1936, 64, 485.

² Menkin, V., J. Exp. Med., 1938, 67, 129.

³ Menkin, V., J. Exp. Med., 1938, 67, 145.

⁴ Menkin, V., Physiol. Rev., 1938, 18, 366; Dynamics of inflammation, 1940, Macmillan Co., New York.

⁵ Menkin, V., and Kadish, M. A., Am. J. Physiol., 1938, 124, 524; Menkin, V., Proc. Soc. Exp. Biol. and Med., 1939, 40, 103.

⁶ Kaiser, P., Schweiz. Z. f. allg. Path. u. Bakteriol., 1939, 2, 1.

on cellular permeability. The studies of Lucké and McCutcheon have demonstrated the usefulness of marine ova as effective material in the study of the living cell in its relation to permeability to water. The rate of passage of water placed in ova in a hypotonic medium is computed from the rate of change of volume. The permeability

is derived from the equation: Permeability = $\frac{d v}{d t}$ /S (P — Pex.) For a detailed description of the terms involved in the equation, the

a detailed description of the terms involved in the equation, the reader is referred to the various publications of Lucké and McCutcheon.^{7–8}

The present studies were made on the eggs of the sea urchin, Arbacia punctulata. For each experiment the ova were obtained from a single specimen of Arbacia and placed in sea water. The diameter of a number of ova was determined with an eyepiece micrometer. The cells are spherical and therefore the diameters were readily converted into measurements of surface area and volume. The ova were then transferred to hypotonic sea water (50%), and the rate of change of volume measured from minute to minute for a period of about 6 minutes. A smooth curve was drawn from which at a given time (2 to 3 minutes) the slope of the curve was obtained by drawing a tangent. Calculation of the permeability to water was then readily computed by applying the above equation. Ova were also exposed for several minutes to sea water containing leukotaxine in concentration of about 5 mg per cubic centimeter. These ova were then transferred to hypotonic sea water (50%) and their rate of swelling immediately measured. In each case the mean volume of several ova was plotted against time. Leukotaxine suspended in sea water induced a slightly acid medium which, after a prolonged interval, seemed to inhibit any change in the permeability of ova. The same type of result was obtained when normal ova were immersed in sea water previously acidified with HCl. For this reason, several experiments were performed by adjusting to a slightly alkaline level with 0.5 N NaOH the pH of the sea water containing leukotaxine. In experiments of short duration, this precaution was found superfluous. It seemed, in brief, as if only ova exposed for several hours to an acid medium failed to swell when subsequently transferred to a hypotonic medium. The data are summarized in Table I. It is clear that leukotaxine-treated ova showed, when immersed in a hypotonic medium, a considerable aug-

⁷ Lucké, B., and McCutcheon, M., Physiol. Rev., 1932, 12, 68.

⁵ Lucke, B., and McCutcheon, M., .frch. Path., 1930, 10, 662.

TAB	LE I.
Effect of Leukotaxine on Permeability o	f Ova to Water When Exposed to Hypo-
tonic Se	a Water.

No. of experiment	Permeability of control ova*	Permeability of leukotaxine-treated ova*	% increase
1	.12	.22	83.3
2	.09	.16	77.7
3	.15	.25	66.6
4	.11	.15	36.4
5	.13	.16	23.1
			
Avg	.12	.19	57.4

^{*}The units of permeability are in terms of cubic micra of water entering per minute, per square micron of cell surface, per atmosphere of pressure.

mentation in their permeability to water. The average increase over that of control ova was about 57%.

The present observations add further support to the view that probably leukotaxine increases capillary permeability by a direct effect on the permeability of the endothelial cell. Bier and Rocha e Silva originally postulated that histamine and leukotaxine were identical substances.9 Their view was severely criticized by the writer.4,5 In view of the mass of accumulated evidence Bier has recently retracted his original contention10 (and personal communication, 1939). His present interpretation, however, that leukotaxine possibly liberates histamine which in turn is responsible for the increased capillary permeability 10 is not supported by any observations. On the contrary, a concentration of histamine (1:20,000 to 1:50,000) equal to that recovered from exudates by Bier and Rocha e Silva, fails to induce the exact pattern of reaction on capillary permeability as elicited by either the untreated exudate or by leukotaxine recovered from such exudative material. It is also of interest to note that histamine fails to augment the permeability of sea urchin ova to water (Lucké, personal communication). In view of the opposite effect obtained with leukotaxine, it would be difficult to postulate that the latter acts on these cells by first releasing histamine. Finally, Ivy has recently succeeded in showing that whereas histamine induces increased gastric secretion of free acid in a Paylov-pouch dog, leukotaxine is wholly ineffective in inducing any such effect (personal communication). This evidence supports further the view that leukotaxine fails to induce a release of

⁹ Bier, O., and Rocha e Silva, M., Arqu. d. Inst. Biologico, 1938, 9, 109, 123, 129. 10 Bier, O., Proc. Third International Congress for Microbiol., New York, 1940, p. 768.

histamine, since the latter in concentration as low as 0.1 mg manifests demonstrable secretion of free acid.

Lucké and McCutcheon showed that Arbacia ova placed in a hypotonic solution display an increased permeability to water. When these cells were returned to ordinary sea water and inseminated, cleavage frequently failed to occur or was atypical.⁸ These investigators expressed the belief that increased permeability is an expression of injury due to the rapid entrance of water. Leukotaxine not only enhances the permeability of sea urchin eggs to water but it also definitely disturbs cleavage development. This would suggest that this substance probably induces a certain amount of cellular injury.

The eggs of Arbacia punctulata were exposed to leukotaxine suspended in sea water in concentration of about 0.7 mg per cubic centimeter. The length of exposure to leukotaxine, prior to insemnation in ordinary sea water, varied from one minute to 3 hours. In some instances, the pH of the sea water containing the leukotaxine-treated ova was adjusted approximately to that of ordinary

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Exp. No.	1.30 min % ova in cleavage	1-3 hr % ova in cleavage	1-30 min % ova in cleavage	1-3 hr % ova in cleavage		
1	42 0	0	40	14		
2	58 92	94 89 58	92	98		
3	32	26 37 6	100	74 76		
4	60	98 66 92	100	100 98		
5	44 58	32	100	94		
6	44 34 18 22		100			
$\Lambda_{\rm V}$		49.75	88.67	79.14		

TABL	E I.
Effect of Leukotaxine on Permeability of	Ova to Water When Exposed to Hypo-
tonic Sea	Water.

No. of experiment	Permeability of control ova*	Permeability of leukotaxine-treated ova*	% increase
1	.12	.22	83.3
2	.09	.16	77.7
3	.15	.25	66.6
4	.11	.15	36.4
5	.13	.16	23.1
			
Avg	.12	.19	57.4

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sea water by the addition of 0.5 N NaOH. This precaution induced essentially no difference in the ultimate results. The effect on cleavage formation obtained in leukotaxine-treated and in control ova is summarized in Table II. In both groups similar containers with approximately the same volume of suspension of eggs were utilized. It is clear that with the exception of one experiment most of the ova in the control group displayed, within several hours, variable numbers of blastomeres. The percentage of ova in cleavage exposed 1 to 30 minutes to sea water prior to insemination averaged 88.67. When exposed for longer intervals (1 to 3 hours) the percentage of ova in various stages of cleavage was slightly reduced, namely 79.14. These two figures stand in sharp contrast with the effect obtained in the leukotaxine-treated group. The number of ova with blastomeres averaged, in the experimental ova, 42 and 49.75% respectively. In other words, about half of the eggs exposed to leukotaxine failed to divide when subsequently inseminated.

Furthermore, the cleavage pattern in the majority of leukotaxine-treated ova appeared abnormal, being characterized by fewer blastomeres than in the control eggs and by unequal forms of division. This is exemplified in Fig. 1 and 2. The former shows normal development of an ovum about one hour and a half following insemination. Fig. 2 illustrates the type of development encountered in an ovum exposed to leukotaxine prior to insemination. The ovum is from the same specimen of *Arbacia* as the one in Fig. 1 and the intervals following insemination are approximately identical in both

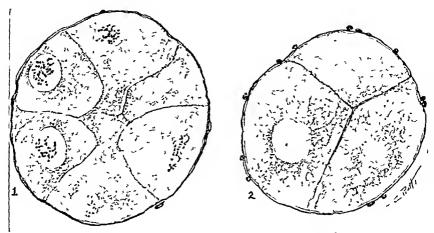


Fig. 1.

Fig. 2.

cases. The illustrations represent histological sections after fixation in Bouin and acetic acid followed by 70% alcohol. This abnormal form of development in the leukotaxine-treated ova was frequently traced back to the fertilization reaction. The fertilization membrane appeared as a zone distinctly narrower than under normal circumstances. It was frequently found surrounded by sperms adhering to it (Fig. 2). Evidence of either cytolysis, absence or localization of the pigment in one area of the ovum were not of infrequent occurrence. These facts suggest that leukotaxine induces some degree of injury to the ova. It is also important to note that besides unequal cleavage, the leukotaxine-treated eggs displayed, after a given interval of time, considerably fewer blastomeres than in the untreated ova (Fig. 1 and 2). This strongly suggests that leukotaxine tends to retard the rate of cleavage.

Finally, sperms exposed for only a few minutes to leukotaxine induced fertilization of the ova of *Arbacia* and the subsequent cleavage pattern appeared to be unaltered. When, however, sperms were placed in contact with leukotaxine for about one hour, there was a sharp reduction in their fertilizing capacity. In some cases 98% of the ova failed to segment. This indicates that leukotaxine is evidently likewise injurious to sperms provided the latter are exposed to this substance for a sufficiently long interval.

Conclusions. Leukotaxine, the substance obtained from inflammatory exudates which is capable per se of increasing capillary permeability and of inducing leukocytic migration, markedly augments the permeability of sea urchin ova to water. Furthermore, a considerable number of ova exposed to this substance manifest abnormal c'eavage development following their insemination. This appears in the form both of unequal cleavage and of an appreciable retardation in the rate of cell division. Sperms are also inactivated after prolonged exposure to leukotaxine. These various manifestations indicate that leukotaxine induces a certain degree of cellular injury when in contact with the ova or sperms of Arbacia punctulata. These effects on invertebrate eggs coupled with its rôle in inflammation suggest that leukotaxine may prove of biological significance in the study of cell division and permeability.

My thanks are due to Doctor B. Lucké for generous advice during the course of this study.

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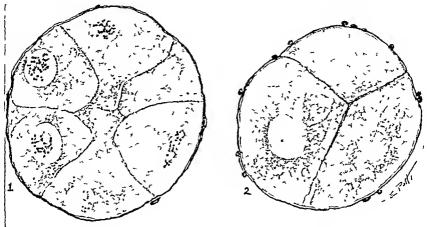


Fig. 1.

Fig 2

was omitted, 4 out of 5 dogs died following ventricular fibrillation when epinephrine was injected during second plane cyclopropane anesthesia. Two test doses of epinephrine had been used in 2 of the cases and only one test dose in the other 2. All 4 of these animals had recovered from previous experiments in which they had been treated with p-amino benzoic acid prior to the injection of the same doses of epinephrine. In the animals that survived the administration of epinephrine alone, the cardiac irregularities were more severe than when the epinephrine injection was preceded by p-amino benzoic acid. One animal in this group developed ventricular fibrillation following the injection of one test dose of epinephrine preceded by p-amino benzoic acid. This animal, however, showed such marked emotional agitation prior to being anesthetized as to suggest the possibility of excess epinephrine secretion being an additive factor to the epinephrine administration.

The action of Paramon was studied in 21 experiments on 9 dogs. The results were similar to those obtained with p-amino benzoic acid. Noteworthy is the fact that when ventricular fibrillation developed in 2 of the animals following the injection of epinephrine alone, the intracardiac injection of 100 mg of procaine in 5 cc saline caused a change from ventricular fibrillation to auricular tachycardia and finally full recovery to sinus rhythm. Subsequently, the intracardiac injection of Paramon under the same conditions in the same animals was ineffective.

Sodium p-amino benzoate which is more soluble than the other 2 drugs permitted the use of larger quantities in less volume of solution. Doses of 10 to 40 mg per kilo were used and found to have effects similar to those of the other 2 dogs. Sixteen experiments on 7 dogs were performed with this drug. Five of the animals showed complete absence of cardiac irregularities when sodium p-amino benzoate was injected before one test dose of epinephrine whereas omission of sodium p-amino-benzoate resulted in ventricular fibrillation in 3 of the animals and ventricular tachycardia in the other 2. The remaining animals showed a few ventricular premature systoles when sodium p-amino benzoate was employed prior to epinephrine in contrast to the development of ventricular tachycardia in one case and ventricular fibrillation in the other when epinephrine alone was administered. In this group also, 2 of the animals that developed ventricular fibrillation were successfully treated by the intracardiac injection of procaine but succumbed when sodium p-amino benzoate was employed at the time of fibrillation.

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Protecting Action of Chemicals Related to Procaine on Ventricular Fibrillation During Cyclopropane Anesthesia.

B. A. MARANGONI, C. L. BURSTEIN AND E. A. ROVENSTINE.

From the Departments of Therapeutics and Anesthesia, New York University College of Medicine, New York City.

In a previous presentation¹ it was reported that procaine reduced the incidence of ventricular fibrillation following the intravenous injection of small doses of epinephrine into dogs during cyclopropane anesthesia. Since the injection of procaine solution into the circulation of man is frequently followed by untoward reactions, it seemed desirable to investigate the action of less toxic substances of the same chemical group. Para-amino benzoic acid, Paramon* and sodium para-amino benzoate were the drugs studied.

Fifty experiments were performed on 21 dogs. Preanesthetic medication, morphine sulphate one mg per kilo and scopolamine hydrobromide 0.04 mg per kilo was injected subcutaneously one hour before each experiment. The carbon dioxide absorption technic was utilized for cyclopropane anesthesia. An unobstructed airway was assured by an endotracheal tube fitted with an inflatable cuff. Depth of anesthesia was maintained at second plane as evidenced by the loss of the lid reflex and maintenance of intercostal activity. Electrocardiograms (lead II) were taken before, during and after drug administration.

The test injection of epinephine was 0.01 mg per kilo in 5 cc of normal saline, given intravenously at the rate of 1 cc per 10 seconds. Para-amino benzoic acid and Paramon were administered intravenously at the dose of 5 to 10 mg per kilo in 20 cc of normal saline injected at the rate of 5 cc in 10 seconds. Sodium p-amino benzoate was administered at the dose of 10 to 40 mg per kilo in 5 cc of normal saline at the rate of 1 cc in 10 seconds.

The effects of p-amino benzoic acid were studied in 13 experiments on 5 dogs. Administration of this drug during cyclopropane anesthesia prior to the injection of epinephrine showed a protecting action against the production of cardiac irregularities. When it

¹ Burstein, Charles L., and Marangoni, Bruno A., Proc. Soc. Exp. Biol. and Med., 1940, 43, 210.

^{*} Paramon is the calcium double salt of benzyl succinic and p-amino benzoic acids prepared and supplied by the Seydel Chemical Company.

was omitted, 4 out of 5 dogs died following ventricular fibrillation when epinephrine was injected during second plane cyclopropane anesthesia. Two test doses of epinephrine had been used in 2 of the cases and only one test dose in the other 2. All 4 of these animals had recovered from previous experiments in which they had been treated with p-amino benzoic acid prior to the injection of the same doses of epinephrine. In the animals that survived the administration of epinephrine alone, the cardiac irregularities were more severe than when the epinephrine injection was preceded by p-amino benzoic acid. One animal in this group developed ventricular fibrillation following the injection of one test dose of epinephrine preceded by p-amino benzoic acid. This animal, however, showed such marked emotional agitation prior to being anesthetized as to suggest the possibility of excess epinephrine secretion being an additive factor to the epinephrine administration.

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Conclusions. The administration of p-amino benzoic acid, the calcium double salt of benzyl succinic and p-amino benzoic acids, or sodium p-amino benzoate prior to a test dose of epinephrine during cyclopropane anesthesia reduced the incidence of ventricular fibrillation. The intracardiac injection of procaine at the time when ventricular fibrillation developed effected a return to normal in a number of cases. Ventricular fibrillation was not ameliorated by the intracardiac injection of the other three p-amino benzoic acid derivatives.

The authors wish to express their appreciation for the helpful suggestions of Dr. Arthur C. DeGraff.

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Anti-Catalase Activity of Sulfanilamide and Related Compounds. VI. Further Studies on Sulfonhydroxamides.

LAWRANCE E. SHINN, EDNA R. MAIN AND RALPH R. MELLON. From The Western Pennsylvania Hospital Institute of Pathology, Pittsburgh, Pa.

In previous studies on the anti-enzymatic concept of the mode of action of sulfanilamide, 1-1 attention has been focused on catalase as one of the enzymes of importance. A time factor was postulated for the conversion of the inactive sulfanilamide to an active anticatalase, which was presumed to result through oxidation to the hydroxylamino derivative. This furnishes an explanation of the characteristic lag period preliminary to the bacteriostatic action of sulfanilamide. It was therefore expected that p-hydroxylamino sulfanilamide, or a similar substance, would exert a bacteriostatic effect without this period of lag and that in addition the action would be more intensive. The sulfonhydroxamides contain a hydroxylamino group which, although located differently in the molecule, contributes anti-catalase activity. Hence they might

¹ Main, E. R., Shinn, L. E., and Mellon, R. R., Proc. Soc. Exp. Biol. and Med., 1938, 39, 272.

² Shinn, L. E., Main, E. R., and Mellon, R. R., Proc. Soc. Exp. Biol. and Med., 1938, 39, 591.

³ Main, E. R., Shinn, L. E., and Mellon, R. R., Proc. Soc. Exp. Biol. and Med., 1939, 42, 115.

⁴ Mellon, R. R., Locke, A. P., and Shinn, L. E., Publication No. 11, A. A. A. S., 1939, pp. 98-113.

behave as preformed active derivatives. It was demonstrated that p-caproylaminobenzenesulfonhydroxamide was capable of initiating bacteriostasis in broth cultures of the pneumococcus without the degree of lag manifested by sulfanilamide and that the bacteriostatic power per mole was more than 4 times as great as that of sulfanilamide. All of the sulfonhydroxamides examined were capable of effecting the accumulation of hydrogen peroxide in pneumococcus cultures.

These results were obtained in the absence of blood. The known reactivity of hydroxylamine and its derivatives for hemoglobin would lead to the expectation that in the presence of blood the hydroxylamino group would be destroyed before reaching the bacterial cell* with a resulting diminution of the bacteriostatic effect. It would not be expected that the immediate nature of the action would be impaired. This bacteriostasis should, however, prove to be transient. There are thus 3 points which can be tested experimentally.

Cultures of a virulent pneumococcus (Neufeld Type I) were established by seeding 1.5 cc samples of defibrinated rabbit blood contained in 1x11 cm tubes with 0.1 cc of a 1/100,000 broth dilution of a 16-hr. blood broth culture (300 organisms) and incubating at 39.5°C for 1.5 hr with intermittent shaking. Plate counts were made at the end of this period and the required compounds added as 0.1 cc of a blood dilution of an alcoholic stock solution. Controls received corresponding amounts of alcohol.† Incubation and shaking were continued and further counts made at 0.75, 1.75, 3.5, and 8.5 hr after the additions. Inhibition was calculated as in the preceding paper.⁵

Fig. 1 A, B, C, F, gives the results of such experiments for p-caproylaminobenzenesulfonhydroxamide and sulfanilamide In order to attain a degree of bacteriostasis comparable with that pro-

⁵ Main, E. R., Shinu, L. E., and Mellon, R. R., Proc. Soc. Exp. Biol. AND Med., 1940, 43, 593.

^{*}Blood containing the sulfoulydroxamides took on a brownish hue during incubation. While no experimental proof of the point is available, this was in all probability the result of methenioglobin formation and an indication of the lability of the hydroxamide group. It has been found in the Sharp and Dohme Laboratories that these sulfoulydroxamides decompose in contact with moisture to yield oxides of nitrogen. Obviously these oxides would tend to produce methemoglobin. They also appear to be responsible for the yellow color produced when broth containing the sulfonhydroxamides was treated with o tolidine.

t The concentration of alcohol in the cultures was 12%. It appeared to have no effect on growth.

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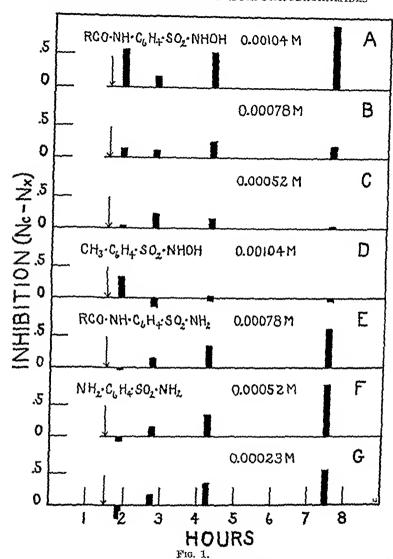
⁴ Mellon, R. R., Locke, A. P., and Shinn, L. E., Publication No. 11, A. A. A. S., 1939, pp. 98-113.

duced by 0.00052 M (9 mg%) sulfanilamide, the concentration of the hydroxamide must be raised to 0.00104 M. This is in contrast to the results for the same compound in broth where less than 0.00016 M was required for the same result.⁵ The expected action of blood in reducing the bacteriostatic efficacy of this type of compound is hence demonstrated. As was anticipated, the bacteriostatic power of the sulfonhydroxamide is established within the first observational period, whereas that of sulfanilamide shows a brief period of stimulation.

The dip in bacteriostatic power at the second interval in Fig. 1 A suggests two diverse actions. The second action appears at about the time that the first is vanishing and resembles that produced by sulfanilamide. This suggests that it can be attributed to a free p-amino group produced from the caproylamino group whereas the transient portion can be referred to the hydroxamide group.

This was tested by employing two other compounds: p-toluene-sulfonhydroxamide and p-caproylaminobenzenesulfonamide in which the two groups are removed to separate molecules. The results are shown in Fig. 1 D and E. When the caproylamino group is absent (D) only the immediate transient action is obtained. When the hydroxamide group is replaced by an amide group (E) no immediate action is found but the second action of p-caproylaminobenzenesulfonhydroxamide is approximately reproduced. Hence it appears that the postulate regarding the prompt but transient nature of the action of the hydroxamide group is correct but that the complete acylaminobenzenesulfonhydroxamide molecule has a double activity. This result made it advisable to investigate the extent of deacylation of caproylamino derivatives in blood.

To whole defibrinated rabbit blood was added p-caproylamino-benzenesulfonhydroxamide or p-caproylaminobenzenesulfonamide to a concentration of 0.00078 M. The samples were incubated at 39.5° and portions withdrawn for analysis by the Marshall method. The results are given in Table I. Both compounds underwent substantial deacylation in blood. In broth no significant deacylation of the sulfonhydroxamide was noted; indicating that deacylation in blood was largely enzymic. That the degree of conversion to free amine in the case of the sulfonhydroxamide is sufficient to account for the bacteriostasis produced is not conclusively demonstrated but in Fig. 1 G is shown the result of experiments with a lower concentration of sulfanilamide (4 mg%) in which very substantial bacteriostasis was produced.



Inhibition of growth of Type I pneumococcus in defibrinated rabbit blood at 39.5°C by: (A) p-caproylaminobenzenesulfonhydroxamide,‡ 0.00104 M; (B) the same, 0.00078 M; (C) the same, 0.00052 M; (D) p-toluenesulfonhydroxamide,‡ 0.0104 M; (E) p-caproylaminobenzenesulfonamide,‡ 0.00078 M; (F) sulfanil amide, 0.00052 M; (G) the same, 0.00023 M. Inhibition is expressed as the difference between the number of generations produced per hour in a control culture (Nc) and in a culture containing the compound added (Nx) over the interval 1.5-2.5, 2.25-3.25, 3.25-5.0, and 5.0-10.0 hr. The values are plotted at the mid-point of the interval concerned. Cultures were inoculated at 0 hours and the compounds added at 1.5 hr (arrow).

[‡] Furnished us through the courtesy of Dr. Maurice Moore of Sharp and Dohme, Inc.

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Bioassay of Water-Soluble Antihemorrhagic Compounds by Intravenous Administration.*

D. RICHERT, SIDNEY A. THAYER, R. W. McKee, S. B. BINKLEY AND EDWARD A. DOISY.

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Since the natural vitamin K compounds are not soluble in water and in many cases superior therapy could be attained by intravenous administration, several different water-soluble antihemorrhagic compounds have been prepared. (Almquist and Klose; Thayer, et al., Fieser and Fry; Foster, et al., and Ansbacher, et al.,).

Supplementing our previous reports (Thayer, et al.), we have assayed 2-methyl-1,4-naphthoquinone and several closely related water-soluble compounds by different methods in order to determine their respective potencies. Originally our products for assay were administered orally but recently the realization that the potencies by parenteral administration might be very different has led us to employ intravenous injection. Compounds which are active orally might be inactive parenterally due to their avoidance of the enzymes of the gastro-intestinal tract or to their too rapid excretion by the kidneys.

The potencies have been estimated from a comparison of the effects of the compounds under investigation and 2-methyl-1,4-naphthoquinone on the, (1) mean prothrombin time, (2) mean clotting time, and, (3) percentage of positive responses.⁷ Each

^{*} We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

¹ Almquist, H. J., and Klose, A. A., J. Am. Chem. Soc., 1939, 61, 1611.

² Thayer, S. A., Binkley, S. B., MacCorquodale, D. W., Doisy, E. A., Emmett, A. D., Brown, R. A., and Bird, O. D., J. Am. Chem. Soc., 1939, 61, 2563; Doisy, E. A., MacCorquodale, D. W., Thayer, S. A., Binkley, S. B., and McKee, R. W., Science, 1939, 90, 407.

³ Fieser, L. F., and Fry, E. W., J. Am. Chem. Soc., 1940, 62, 228.

¹ Foster, R. H. K., Lee, J., and Solmssen, U. V., J. Am. Chem. Soc., 1940, 62, 453.

⁵ Ansbacher, S., Fernholz, E., and Dolliver, M. A., PROC. Soc. Exp. Biol. And Med., 1940, 43, 652.

⁶ Thayer, S. A., Cheney, L. C., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., J. Am. Chem. Soc., 1939, 61, 1932.

⁷ Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., Proc. Soc. Exp. Biol. and Mfd., 1939, 41, 194.

TABLE I.

Deacylation of p-Caproylaminobenzenesulfonhydroxamide aud p-Caproylaminobenzenesulfonamide in Broth and Defibrinated Rabbit Blood at 39.5°C.

		A		В		
Hr	% deacylatiou in broth pH 7.4	% deaeylation in blood	mg% sulfanilauide equivalent in blood	% deacylation in blood	mg% sulfanilamide equivalent in blood	
0	1.3	0.8	0.1	0.4	0.05	
0.75		3.6	0.5	4.1	0.5	
1.75		5.8	0.8	7.5	1.6	
3.50	1.3	10.8	1.5	18.0	2.7	
6.75	-	15.7	2.1	32.0	4.3	
24.00		29.8	4.0	76.0	10.2	

A. p-caproylaminobeuzeuesulfonhydroxamide 0.00078 M.

B. p-caproylaminobenzenesulfonamide 0.00078 M.

Discussion. p-Caproylaminobenzenesulfonhydroxamide appears to represent an approach to that hypothetical compound which shall be "preformed" in the sense of possessing the group or groups necessary to bacteriostatic activity which are normally formed by the micro-organism itself. That the present compound is not ideal is demonstrated by the transient nature of the powerful early effect. The demonstration of two successive activities arising from the same molecule opens interesting fields of speculation and experiment regarding future objectives in the production of better therapeutic agents.

Summary. p-Caproylaminobenzenesulfonhydroxamide produces bacteriostasis of pneumococci in blood without the lag characteristic of sulfanilamide. The activity is only about one-eighth of the corresponding bacteriostatic power in broth. That the immediate nature of the effect is due to the hydroxamide group was demonstrated by the use of p-toluenesulfonhydroxamide in which the potentially active p-amino group is absent. The effect of the hydroxamide group is transient in nature. A second period of bacteriostatic activity manifested by p-caproylaminobenzenesulfonhydroxamide is probably due to the free amino group formed by deacylation of the caproylamino group.

11540

Bioassay of Water-Soluble Antihemorrhagic Compounds by Intravenous Administration.*

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From the Depurtment of Biochemistry, St. Louis University School of Medicine, St. Louis.

Since the natural vitamin K compounds are not soluble in water and in many cases superior therapy could be attained by intravenous administration, several different water-soluble antihemorrhagic compounds have been prepared. (Almquist and Klose; Thayer, et al., Fieser and Fry; Foster, et al., and Ansbacher, et al.,).

Supplementing our previous reports (Thayer, ct al.), we have assayed 2-methyl-1,4-naphthoquinone and several closely related water-soluble compounds by different methods in order to determine their respective potencies. Originally our products for assay were administered orally but recently the realization that the potencies by parenteral administration might be very different has led us to employ intravenous injection. Compounds which are active orally might be inactive parenterally due to their avoidance of the enzymes of the gastro-intestinal tract or to their too rapid excretion by the kidneys.

The potencies have been estimated from a comparison of the effects of the compounds under investigation and 2-methyl-1,4-naphthoquinone on the, (1) mean prothrombin time, (2) mean clotting time, and, (3) percentage of positive responses.⁷ Each

^{*} We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

¹ Almquist, H. J., and Klose, A. A., J. Am. Chem. Soc., 1939, 61, 1611.

² Thayer, S. A., Binkley, S. B., MacCorquodale, D. W., Doisy, E. A., Emmett, A. D., Brown, R. A., and Bird, O. D., J. Am. Chem. Soc., 1939, 61, 2563; Doisy, E. A., MacCorquodale, D. W., Thayer, S. A., Binkley, S. B., and McKee, R. W., Science, 1939, 90, 407.

³ Fieser, L. F., and Fry, E. W., J. Am. Chem. Soc., 1940, 62, 228.

⁴ Foster, R. H. K., Lee, J., and Solmssen, U. V., J. Am. Chem. Soc., 1940, 62, 453.

⁵ Ausbacher, S., Fernholz, E., and Dolliver, M. A., Proc. Soc. Exp. Biol. And Med., 1940, 43, 652.

⁶ Thayer, S. A., Cheney, L. C., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., J. Am. Chem. Soc., 1939, 61, 1932.

⁷ Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A., Proc. Soc. Enp. Biol. and Med., 1939, 41, 194.

TABLE I.
Bioassay of Water Soluble Compounds (Intravenous Administration)

Compounds	Dosnge, µg	Equivalent in µg of standard,†	Chicks used (21 days of age) No.	Response normal clotting time,	Clotting time, mean; S.E.,	Prothrombin time, mean; S.E.,
1,4-dihydroxy-2-methylnaphthalene monosuccinate 2-methyl-1,4-naphthoquinone (standard) Controls lot 1	0.751 f 1.502 0.961 1.432	0.68	20000000000000000000000000000000000000	5.8 9.3 80 100 0	17.8 ± 6.8 6.7 ± 0.5 12.7 ± 4.9 6.0 ± 0.8 129.7	200 41.9 ± 4.9 29.1 ± 2.2 35.7 ± 4.1 58.0 50.6
di-potassium 1,4 dihydroxy-2- methylnaphthalene-disulfate 2 methyl-1,4-naphthoquinone (standard) Controls lot 3	2.503 6.000 6.005 0.963	1.0 2.3.3 3.3.3	41 12 10 10 10	64 71 71 100 0	29.1 ± 6.4 7.0 ± 0.82 8.9 ± 1.1 9.9 ± 5.6 4.7 ± 0.7	44.3 ± 8.6 33.5 ± 0.48 37.1 ± 1.50 28.6 ± 1.2
4.amino-2.methyl-1.naphthol* 2.methyl-1,4.naphthoquinone (standard) Coutrols lot 4	1.501	0.74 1.1 1.0	29 15 20 10	89 93 100 0	6.7 7.0 + 1.4 7.1 + 1.4 7.1 + 1.9 6.1 + 1.9 1.0.90	91.2 27.8 27.8 11.2 27.4 11.5 27.4 11.6
4-amino-3-methyl-1-naphthol* 4-amino-2-methyl-1-naphthol* Controls lot 5 Normal chicks	1,305	1.0	15 10 10	88 E 0 0	5.7 ± 0.65 8.4 ± 1.4 296.6	$\begin{array}{c} 60.6 \\ 32.5 \pm 1.2 \\ 35.5 \pm 1.30 \\ 67.8 \end{array}$
As the crystalline hydrochloride containing 0.5 M otherol.	taining 0.5 f chicks use	M ethanol.			4.0 H 0.1	26.5 ± 1.0

1.4-naphthoquinone and the respective compound.

assay has been controlled by an accompanying assay of a standard substance (usually 2-methyl-1,4-naphthoquinone) on the same lot of deficient chicks to eliminate the variation in deficiency found in different groups of chicks.

The basal diet used in all of our experiments is the one described

by Almquist.

Assays—The compounds were dissolved in 0.85% NaCl solution. The chicks were slightly anesthetized with ether and the solution was injected directly into the jugular vein of the chick (0.1 cc of solution). Bleeding was minimized by the use of a fine needle (27 gauge) and by entering the vein through the subcutaneous tissues. Eighteen hours after the injection blood was drawn from the brachial vein into a small tube (micro test tube of Fischer)^s and placed at once in a thermostat adjusted to 38.5-39.5°C and the time for coagulation determined. The chick's head was clipped off with scissors and 1.8 cc of blood collected in a vial containing 0.20 cc of 0.1 M sodium oxalate solution. The method of Almquist and Klose⁹ was used for determining prothrombin time.

Early in our work on Vitamin K, we realized that the variability of the degree of deficiency of different lots of chicks (see values for the controls Table I) could produce gross inaccuracies in the bioassay. Originally we standardized the deficiency of each lot of chicks by determining the response to 2 different dosages of a stock solution which had been obtained from alfalfa. Later,² we suggested the adoption of pure crystalline 2-methyl-1,4-naphthoquinone as the basic standard. The variability of the response of these lots of chicks (1, 3 and 6) to the administration of 0.96 μ g of 2-methyl-1,4-naphthoquinone (Table I) emphasizes the necessity of ascertaining the response of each lot of chicks to a standard.

On a weight basis, 1,4-dihydroxy-2-methylnaphthalene monosuccinate, 4-amino-2-methyl-1-naphthol and 4-amino-3-methyl-1-naphthol administered intravenously are, perhaps, slightly less active than 2-methyl-1,4-naphthoquinone; on a molar basis all are fully as active. The potency of the disulfate on a weight basis is somewhat less than one-sixth and on a molecular basis approximately one-third that of 2-methyl-1,4-naphthoquinone.

Summary. Using intravenous administration it has been found that on a molecular basis the potencies of all the compounds with

S Fischer, A., Pfluger's .trch. ges. Physiol., 1930, 225, 737.

⁹ Almquist, H. J., and Klose, A. A., Biochem. J., 1939, 33, 1055.

the exception of the disulfate are approximately equal to that of the standard, 2-methyl-1,4-naphthoquinone. The disulfate is about one-third as potent on a molecular basis, but owing to the much larger molecular weight its activity per milligram is somewhat less than one-sixth that of 2-methyl-1,4-naphthoquinone.

11541 P

Riboflavin Determinations on Normal Liver and Liver Tumor.

HERBERT KAHLER AND EVERETT F. DAVIS. (Introduced by Carl Voegtlin.)

From the National Cancer Institute, U. S. Public Health Service.

It was decided to use a fluorometric method in making quantitative estimations of riboflavin from fresh liver tissue after several checks had been run against the Snell and Strong method.* The fluorometric method here used is first to determine the total fluorescence, then eliminate the riboflavin by raising the alkalinity to pH 11 and determining the "interfering" fluorescence. The difference between these two values gives the approximate riboflavin value. The fluorescence was measured with a photocell using suitable optical filters.

Liver tumors from Osborn-Mendel rats which had been fed 2-amino 5-azo toluene for a long period were used. These animals were kindly put at our disposal by Dr. E. Emmart. Samples of liver which had been perfused with saline were ground with n/10 HCl, autoclaved for 15 min. and clarified by precipitation at pH 5-6, followed by filtering through No. 42 paper. Recovery of added riboflavin was about 97% by this fluorometric method.

Some of the material was given a short low temperature drying by the lyophile process eliminating most of the water. The data in the tables indicate that the difference between normal and tumor liver is not due to water content.

Comparisons were made between normal livers from normal rats, nontumor bearing liver from dye-fed rats, liver tumors from dye-fed rats, residual liver from which the tumor had been excised, fetal liver, and leg muscle. In the case of a general riboflavin deficiency it would be expected that lower riboflavin values for the muscle would be ob-

^{*} Made by Drs. Isbell and Wooley of the National Institute of Health.

TABLE I.
Riboflavin Concentration in Micrograms per Gram of Fresh Tissue.

		7/		
	Histologic		Liver	Leg muscle,
Rat No.	diagnosis on liver	Tumor	residue	~/g
1	Tumor (no diagnosis)	S	16	
2	,, `,, ~,,	12	15	
2 3 4	Carcinoma	11	15	
4	Pooled careinoma, hepatoma	16	16	
5*	Carcinoma, Region A	15.7		2.52
-	, , B	13.2		
	{B {C	18.6		
6*	Hepatoma	19.2	20.7	
7	",	21.3	21.3	
6* 7 8 9	**	16.9	24.7	
9	,, careinoma	99.9	22.2	2.80
10	'' , eirrhosis	25.7	29.8	2.95
11	, carcinoma			
	+ cirrhosis	26.8	27.95	2,56
D	ye fed; no tumors	y/g		
12	Cirrhosis	26.14		2.1
13	'' (slight)	27.84		2.9
14	,, ',,' '	29.8		3.3
15	"	31.04		
16	11	34.0		2.1
17	Essentially normal	35.7		
7.	formal rats, normal diet			
18	Normal	25.0		
19		25.5		
20		25.85		
21		28.6		
20		30.S		
23		30.9		
24		39.0		
25	Normal fetal liver	4.7		
26	" " "	6.3		

^{*}Large amount of blood in tissue.

TABLE II.
Riboflavin Concentration in Micrograms per Gram of Dried Tissue.

Rat No.	Histology	y/g dry wt	y/g wet wt (calc.)
27, 28 29, 30	Pooled material, carcinoma and hepatoma	58.9	14.9
29, 30 31	Pooled hepatoma, carcinoma	00.9	14.9
	and cirrhotic liver	59.4	15.0
32	Normal	106,6	29.0
33	> ,	106.8	29.0

tained. A value of $1.4 \text{ } \gamma$ is reported for adult rat muscle on a deficient diet.

¹ Fraser, H., Topping, N., and Isbell, H., Public Health Rep., 1940, 33, 280.

the exception of the disulfate are approximately equal to that of the standard, 2-methyl-1,4-naphthoquinone. The disulfate is about one-third as potent on a molecular basis, but owing to the much larger molecular weight its activity per milligram is somewhat less than one-sixth that of 2-methyl-1,4-naphthoquinone.

11541 P

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Liver tumors from Osborn-Mendel rats which had been fed 2-amino 5-azo toluene for a long period were used. These animals were kindly put at our disposal by Dr. E. Emmart. Samples of liver which had been perfused with saline were ground with n/10 HCl, autoclaved for 15 min. and clarified by precipitation at pH 5-6, followed by filtering through No. 42 paper. Recovery of added riboflavin was about 97% by this fluorometric method.

Some of the material was given a short low temperature drying by the lyophile process eliminating most of the water. The data in the tables indicate that the difference between normal and tumor liver is not due to water content.

Comparisons were made between normal livers from normal rats, nontumor bearing liver from dye-fed rats, liver tumors from dye-fed rats, residual liver from which the tumor had been excised, fetal liver, and leg muscle. In the case of a general riboflavin deficiency it would be expected that lower riboflavin values for the muscle would be ob-

^{*} Made by Drs. Isbell and Wooley of the National Institute of Health.

TABLE I. Riboflavin Concentration in Micrograms per Gram of Fresh Tissue.

		/ب-	'g	
Rat No.	Histologic diagnosis on liver	Tumor	Liver residue	Leg muscle,
1	Tumor (no diagnosis)	S	16	
2	,, `,, `,, *	12	15	
2 3 4	Careinoma	11	15	
4	Pooled carcinoma, hepatoma	16	16	
5*	Carcinoma, Region [A	15.7		2.52
	↓ B ↓ C	13.2		
	į C	18.6		
6*	Hepatoma	19.2	20.7	
6* 7 8 9	~ 1,	21.3	21.3	
8	"	16.9	24.7	
	'' earcinoma	22.2	22.2	2.80
10	'' , eirrhosis	25.7	29.8	2.95
11	'' , earcinoma			
	+ cirrhosis	26.8	27.95	2.56
)	Oye fed; no tumors	y/g		
12	Cirrhosis	26.14		2.1
13	'' (slight)	27.84		2.9
14	,, `,;´´	29.S		3.3
15	"	31.04		
16	"	34.0		2.1
17	Essentially normal	35.7		
	Normal rats, normal diet			
18	Normal	25.0		
19		25.5		
20		25.85		
21		28.6		
22 23		30.8		
23		30.9		
24		39.0		
25	Normal fetal liver	4.7		
26	" "	6.3		

^{*}Large amount of blood in tissue.

TABLE II.
Ribotlavin Concentration in Micrograms per Gram of Dried Tissue.

Rat No.	Histology	i/g dry wt	y/g wet wt (calc.)
27, 28 29, 30	Pooled material, earcinoma and hepatoma	70.0	
31	Pooled hepatoma, carcinoma	58.9	14.9
	and eirrhotic liver	59.4	15.0
32	Normal	106.6	29.0
33	"	106.S	29.0

tained. A value of 1.4 γ is reported for adult rat muscle on a deficient diet.

¹ Fraser, H., Topping, N., and Isbell, H., Public Health Rep., 1940, 55, 250.

In the absence of any other criteria the estimation of "malignancy" was based solely on histologic grounds. The term hepatoma was used to denote growths which reproduced the normal liver structure so closely that there was no histologic indication of "malignancy". On the other hand certain of these tumors manifested such extreme degrees of deviation from normal liver cell structure and architecture that they were termed carcinomas to denote their malignant histologic appearance.

The following conclusions may be drawn from these results:

- 1. The average value for normal and cirrhotic livers of 30.1 γ (age 256 to 550 days) agrees with Fraser, Topping and Isbell's values for considerably younger rats of 33 γ (age 130 days).
- 2. Continuously dye fed rats with cirrhosis not developing gross tumors gave normal riboflavin content for liver.
- 3. Livers in which tumors had developed gave a lower value per gram of fresh tumor tissue and per gram of residual tissue than normal liver.
- 4. On the basis of lyophilized dry weight normal liver is considerably higher in riboflavin concentration than tumor material.
- 5. The muscle values indicate that the animal is not suffering from a general riboflavin deficiency.

These results are consistent with the earlier findings² that lactic acid accumulates in various tumors especially following large sugar injections. According to current views³ pyruvic acid which is formed from sugar break down is normally oxidized to CO₂ and H₂O through molecular oxygen and the flavoprotein-cytochrome enzymes. With a deficient oxygen and/or enzyme concentration the pyruvic acid is converted to lactic acid giving tumors a low pH. In this connection see the recent finding of low coenzyme in tumor⁴ and the high ratio of reduced to oxidized cozymase in Jensen sarcoma.⁵

The histologic diagnoses were made by Drs. H. L. Stewart and Hugh G. Grady.

² Voegtlin, C., Fitch, R., Kahler, H., Johnson, J. M., and Thompson, J. W., Nat. Inst. Health Bull., No. 164, 1935.

³ Eric Ball, Johns Hopkins Hosp. Bull., 1939, 65, 253.

⁴ Bernheim, F., and Felsovanyi, A. V., Science, 1940, 91, 76.

⁵ Euler, H. V., Schlenk, F., Heiwinkel, H., and Högberg, B., Z. f. physiol. Chem., 1938, 256, 208.

11542

Absorption of Water-Soluble Vitamin K from Intestinal Tract.*;

E. D. WARNER AND JOSEPH E. FLYNN.

From the Department of Pathology, State University of Iowa, Iowa City, Iowa.

Bile salts are known to be essential for the absorption of fatsoluble forms of vitamin K from the intestinal tract, but it is not
known whether they are of benefit in the absorption of the watersoluble forms. To obtain data regarding this, we have used bileobstructed rats in which the vitamin K reserves were depleted preoperatively by the technic previously reported from this laboratory. After ligation of the bile duct, the animals were placed on
a diet from which vitamin K was still more rigidly excluded.
Within 3-4 days the prothrombin level falls into the bleeding zone.
The subsequent rise in prothrombin, following the oral administration of vitamin K, was used as a measure of the extent to which
the vitamin is utilized, either with or without supplements of bile
salts.

Prothrombin determinations were made by the 2-stage technic of Warner, Brinkhous, and Smith;^{3,4} bile salts (1 cc of 3% sodium taurocholate) and vitamin K were given through a metal tube into the stomach. As a source of water-soluble vitamin K, we used the potassium salt of the disulfuric acid ester of 2-methyl-1,4-naphthohydroquinone. Since this work was initiated the sodium salt of this compound was described by Fieser.⁵ It is apparently somewhat less potent⁶ than 2-methyl-1,4-naphthoquinone, when the two

^{*} Aided by a grant from the John and Mary R. Markle Foundation. Funds for assistance were also supplied by the Graduate College, State University of Iowa.

f The potassium salt of the disulfuric acid ester of 2-methyl-1,4-naphthohydroquinone, used in these experiments, was prepared in November, 1939, as a part of a cooperative program in which a series of compounds of this type was synthesized by George H. Coleman, J. J. Carnes and D. W. Kaiser of the Department of Chemistry, State University of Iowa.

¹ Flynn, Joseph E., and Warner, E. D., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 190.

² Tidrick, Robert T., Joyce, Frank T., and Smith, H. P., Proc. Soc. Exp. Biol. and Med., 1939, 42, 853.

³ Warner, E. D., Brinkhous, K. M., and Smith, H. P., Am. J. Physiol., 1936, 114, 667.

Smith, H. P., Warner, E. D., and Brinkhous, K. M., J. Exp. Med., 1937, 66, 801.

⁵ Fieser, L. F., and Fry, E. M., J. Am. Chem. Soc., 1940, 62, 228.

⁶ Ansbacher, S., Fernholz, E., and Delliver, M. A., PROC. Soc. Exp. Biol. And Med., 1940, 43, 652.

					TABLE I.				
Response	οf	Rats*	to	a	Water-soluble	Form	of	Vitamin	K.

	Prothrom	Prothrombin levels (% of normal)					
Days of treatment	2 y of vitamin daily	5 γ of vitamin daily	8 y of vitamin daily				
Resul	ts with vitamin K	plus bile salt	S.	~~~~			
0	24	19	17				
1	14	52	57				
2	dead t	52	78				
			106				
Results v	vith vitamin K bu	t without bile	salts.				
0	15	12	29				
1	14	16	50				
2	15	38	86				
3		55					
4		68	91				

compounds are compared on a molar basis. Our own experience indicates that the compound is non-toxic when given orally to rats in doses 100 times the physiological requirements.

Results. Table I shows typical examples of the response of Kdeficient rats following administration of daily doses of 2, 5 and 8 µg of the water-soluble compound. It is readily seen that bile salt did not appreciably modify the therapeutic efficacy of the vitamin. At the level of 2 µg, the prothrombin level remained in the bleeding zone. At the level of 5 µg, the recovery was 35-70% complete, both with and without bile salt, and with daily doses of 8 μ g the prothrombin rose above the 75% level.

When fat-soluble forms of vitamin K are administered to man, the danger always exists that the dose of bile salt will not be adequate in amount, or that the bile salt may not mingle properly with the vitamin following dissolution of the capsules. The oral administration of water-soluble vitamin K, if effective in man, will eliminate this problem. It will also eliminate much of the nausea and vomiting produced by the bile supplements. It is obvious that when the acute phase of the disease subsides, bile salt should then be given to enable absorption of the many fat-soluble components of the diet.

^{*} Albino rats weighing 200 to 300 g.
† Autopsy showed extensive intraübdominal hemorrhage.

11543

Effect of Vitamin K on Hypoprothrombinemia of Experimental Liver Injury.*

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From the Department of Pathology, State University of Iowa, Iowa City.

It has been shown that the plasma prothrombin level declines when the liver is injured^{1, 2, 3} or when it is excised, either in part⁴ or in toto.^{5, 8} Hypoprothrombinemia develops also with vitamin K deficiency. In man, the two factors are often combined and the response to vitamin K is commonly incomplete. In such patients, the extent to which vitamin K compensates for the liver injury, if at all, is difficult to determine. To study this question further, we have performed experiments to determine the influence of vitamin K in excess on the hypoprothrombinemia which develops following liver injury alone.

Liver injury was produced in dogs (10-15 kg) by repeated administration of small doses of chloroform, as described previously.² In each experiment, 2 dogs of the same weight were given identical diets (mixed table scraps) and identical doses of chloroform. In addition, one of the animals of each pair received a daily vitamin K supplement consisting of the petroleum ether extract of 200 g alfalfa meal, emulsified in 30 cc of 2% solution of Wilson's bile salt. Plasma prothrombin determinations were made by the method of Warner, Brinkhous and Smith.^{1,2}

Almost identical results were obtained in each of the 4 experiments performed. Chart 1 shows a typical experiment. It is seen that the administration of vitamin K failed to modify in any way, either the fall in prothrombin with chloroform administration or the rise in prothrombin during the recovery period.

In another experiment the daily vitamin supplement was started

^{*} Aided by a grant from the John and Mary R. Markle Foundation. Funds for technical assistance were supplied by the Graduate College, State University of Iowa.

¹ Warner, E. D., Brinkhous, K. M., and Smith, H. P., Am. J. Physiol., 1936, *114, 667.

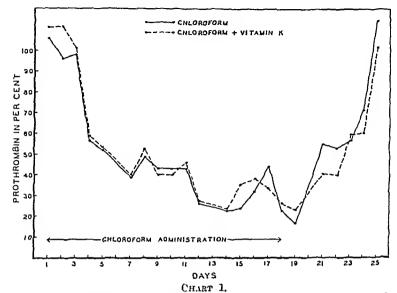
² Smith, H. P., Warner, E. D., and Brinkhous, K. M., J. Exp. Med., 1937, 66, 801.

³ Quick, A. J., J. A. M. A., 1938, 110, 1658.

⁴ Warner, E. D., J. Exp. Med., 1938, 68, 831.

⁵ Warren, R., and Rhoads, J. E., Am. J. Med. Sci., 1939, 198. 193.

⁶ Andrus, W. D., Lord, J. W., and Moore, R. A., Surgery, 1939, 6, 899.



The consecutive daily doses of chloroform given during the 18-day period of chloroform administration were 2, 2, 3, 2, 3, 2, 3, 31/2, 31/2, 41/2, 5, 5, 4, 5, 6, 6, 7 and 6 cc.

one week prior to the beginning of chloroform administration. This procedure likewise had no demonstrable effect on the fall in plasma prothrombin.

It is suggested that when vitamin K deficiency and liver injury are both present, as in many patients, administration of the vitamin may correct the former, but one cannot expect that an excess of the vitamin will compensate for the element of liver injury.

Summary. The hypoprothrombinemia, which develops following liver injury (chronic chloroform intoxication), is not influenced by vitamin K administration.

115H P

Study of Certain Tissue Lipids in Generalized Lipodystrophy ("Lipohistiodiaresis").*

ARILD E. HANSEN AND IRVINE McQUARRIE.

From the Department of Pediatrics, University of Minnesota, Minneapolis.

An unusual opportunity to study the lipid composition of various. tissues in an extremely rare condition, that of generalized lipodystrophy, was offered when death occurred in a 9-year-old boy in whom an almost complete absence of adipose tissue from the body had been present for the past 6 years (Case 1). In addition to this remarkable apparent lack of body fat, the symptom complex was composed of cirrhosis of the liver, chronic fibrosis of the spleen, pancreas and certain lymph nodes, and diabetes mellitus. Necropsy was begun within one hour following demise, at which time samples of various tissues were obtained. After being weighed, the specimens (usually about 1 g of tissue) were placed in 95% alcohol and allowed to stand for 24 hours. The tissues were then ground with sea sand in a mortar, rinsed several times with alcohol and ether, returned to the original flasks, and sufficient ether added to make approximately a 3:1 alcohol-ether mixture. The flasks were immersed in a boiling water bath for about 5 minutes and allowed to cool; the contents were filtered through fat-free filter paper into volumetric flasks, brought to volume, and stored in a refrigerator until analyses were made. Aliquots were measured and the following procedures employed: The method of Wilson and Hansen¹ was used for the determination of the unsaponifiable and saponifiable fractions, while the technic followed by Hansen² was used for the determination of the fatty acids in the acetone-insoluble (phospholipid) fraction and the acetone-soluble (cholesterol esterneutral fat) fraction. The total cholesterol and cholesterol esters were determined by the procedure described by Bloor,3,4 the photoelectric colorimeter being used in obtaining the final readings. For the control studies, similar tissues from a 14-year-old boy dving in

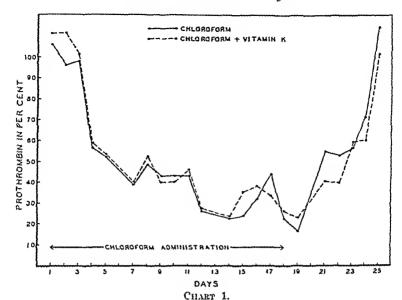
^{*} Aided by grants from Mead Johnson and Company and the Medical Graduate Research Fund of the University of Minnesota.

¹ Wilson, Wm. R., and Hansen, Arild E., J. Biol. Chem., 1936, 112, 457.

² Hansen, Arild E., Proc. Soc. Exp. Biol. and Med., 1939, 40, 376.

³ Bloor, W. R., J. Biol. Chem., 1916, 24, 227.

⁴ Bloor, W. R., and Knudson, A., J. Biol. Chem., 1916, 27, 107.



The consecutive daily doses of chloroform given during the 18-day period of chloroform administration were 2, 2, 3, 2, 3, 2, 3, $3\frac{1}{2}$, $3\frac{1}{2}$, $4\frac{1}{2}$, 5, 5, 4, 5, 6, 6, 7 and 6 cc.

one week prior to the beginning of chloroform administration. This procedure likewise had no demonstrable effect on the fall in plasma prothrombin.

It is suggested that when vitamin K deficiency and liver injury are both present, as in many patients, administration of the vitamin may correct the former, but one cannot expect that an excess of the vitamin will compensate for the element of liver injury.

Summary. The hypoprothrombinemia, which develops following liver injury (chronic chloroform intoxication), is not influenced by vitamin K administration.

total fatty acid values of the skin in Case 1 are greatly diminished when compared with those of the control subject (Case 2). In spite of the great difference in the amount of fat present in this tissue, we find that the qualitative characteristics of the fatty acids as regards average molecular weight and average iodine number are practically the same in both instances.

That there is a definite lack of fatty acids in these tissues becomes even more striking when we consider the calculated values for the neutral fatty acids. These calculations disclose that the approximate values for the neutral fat fatty acids in the hepatic tissue of our control subject (Case 2) are 5 times that of those for the child with the generalized lipodystrophy, while those in the skin are 50 times that of those in Case 1. Even more striking is the finding that the tissues in the perirenal region of Case 2 contained almost 400 times as much neutral fat as those in Case 1. From these data, it appears that there is a marked lack of fatty material in the body of the child with generalized lipodystrophy, which confirms the clinical and pathological (gross and microscopic) diagnosis. Further, we may conclude that this deficiency apparently is specifically due to a lack of neutral fat fatty acids from the various tissues studied. The name "lipohistiodiaresis" (lack of fat in the tissues) has been suggested to describe this phase of the condition.

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Increased Serum Phosphatase Activity Without Hyperbilirubinemia after Ligation of Hepatic Ducts in Dogs.

ALEXANDER B. GUTMAN, BRUCE M. HOGG AND KENNETH B. OLSON.*

From the Departments of Medicine and Surgery, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

In man with complete obstruction of the common bile duct, hyperbilirubinemia is associated with markedly increased phosphatase activity of the serum. When obstruction of the common duct is incomplete (as frequently in choledocholithiasis, cholangeitis) or in intrahepatic biliary tract obstruction (hepatic metastases, etc.), little or no jaundice may result but the serum phosphatase is usually

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TABLE I.

Lipid Composition of Various Tissues in Child with Generalized Lipedystrophy

(Case 1—D.Z.) and in Child with Subacute Nephritis (Case 2—G C.).

	Perirenal		Subcutaneous	Liver		Skin	
Lipid fraction	Case 1	Case 2	Case 2	Case 1	Case 2	Case	1 Case 2
Unsap. fraction*	459	902	691	1,197	615	306	316
Total fatty acids							
Sap.*	452	44,610	51,144	1,490	3,156	340	10,025
M.W.	292		279	204	290	276	274
I,N.	102	62	66	107	102	68	64
Acet. Sol. F. A.							
Sap.*	214	36,075	50,076	697	1,498	209	10,034
M.W.	268	274	278	293	281		274
I.N.	86	60	65	83	74	68	64
Acet. Insol. F.A.							
Sap.*	244	199	309	797	1,599	127	160
M.W.	294			301	296		
I.N.	127		-	123	121		~~
Cholesterol							
Total*	296	242		893	356	162	102
Ester*	83	194		329	77	29	3'3
Neutral Fat F. A.* (Calc.)	130	44,320	50,000	360	1,500	190	10,010

^{*}Expressed in mg per 100 g wet tissue.

uremia from subacute nephritis (Case 2), the best available material at the time, were used.

The results are summarized in Table I.

It is readily apparent from inspection of the data in Table I that the total fatty acids of the various tissues are distinctly less in the child with the generalized lipodystrophy (Case 1) than in the control subject (Case 2). In the perirenal tissues, the unsaponifiable fraction and the cholesterol esters are also less in Case 1, while the values for the acetone-insoluble (phospholipid) fatty acids are essentially the same in both cases. As regards the subcutaneous material, it was impossible to find any adipose tissue in Case 1 which was suitable for analysis. In the liver, the value for the total fatty acids in Case 1 is but one-half that of Case 2, while the unsaponifiable fraction as well as the total cholesterol and cholesterol ester values are higher in this child with lipodystrophy. The acetone-insoluble (phospholipid) fatty acids in this tissue are definitely less in Case 1. As regards the skin, it is interesting to note that the values for the unsaponifiable fraction, total cholesterol, cholesterol esters, and the acetone-insoluble (phospholipid) fatty acids are essentially the same in both cases. On the other hand the

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and Armstrong method) and bile. The animals were sacrificed after 4-9 weeks and the presence of intact ligatures with dilatation of the proximal hepatic ducts verified at postmortem.

Control preoperative serum and urine analyses were obtained. Nembutal anesthesia was found not to affect serum phosphatase values. Urine samples were obtained by catheter to avoid contamination with phosphatase- and bile-rich feces.

Results. The serum phosphatase activity increased within 24 hours after operation to reach 5-20-fold levels in 4 days and a peak usually between the first and second week; the maximum level varying with the number of hepatic ducts ligated. A spontaneous decline followed, with return to approximately normal levels after some 2 months. A roughly parallel rise in the total cholesterol content of the serum was noted though the return to normal was more rapid. No significant increase in serum bilirubin occurred except a transient rise to 0.5 mg % on the fourth day in Dogs 1 and 3. Bile pigments appeared in the urine within 24 hours after operation and persisted for many weeks, gradually decreasing in amount. Significant "alkaline" phosphatase activity was not present in any urine specimens with the exception of small amounts in the urine of Dog 3. At postmortem, the liver lobes tied off appeared to be normal grossly and showed only slight changes histologically.

Discussion. It is not clear whether the dissociation of serum phosphatase and bilirubin levels observed clinically with incomplete biliary obstruction and reproduced in these experiments should be regarded as incompatible with the "phosphatase retention" theory—that the increase in serum phosphatase levels in obstructive jaundice is due to retention of phosphatase normally excreted in the bile. One factor that might cause such dissociation in blood levels following

TABLE I.

Phosphatase Activity (P, in Bodansky units/100 cc) and Total Cholesterol (C, in mg/100 cc) of the Serum after Ligation of Hepatic Ducts in the Dog.

Time post-	⅓ liver	g 1 ligated C	Do 1½ liver P	g 2 ligated C	Do 1/2 liver P		Dog ¼ liver P	
Pre-op.	3.7 7.3	120 172	4.1	154	3.4	138	2.8	176
4 '' 1 wk	26.2	171	$\frac{19.6}{21.7}$	182 247	61.7	250	29.4	235
1½ " 2½ "	$16.9 \\ 11.2$	192 117	22.1 9.9	154	74.0	435	0= 1	007
4 "	6.8	111	6.8	149	49.1 28.2	268	25.1 13.4	$\begin{array}{c} 207 \\ 205 \end{array}$
5 '' 7 ''	4.4				14.7 9.2	$195 \\ 149$	7.8 7.2	169
9 ,,					4.5	110	3.7	138

distinctly elevated. In hepatitis ("catarrhal" jaundice), even marked hyperbilirubinemia is associated quite regularly with comparatively little rise in serum phosphatase activity.

Corresponding changes in serum phosphatase have been produced experimentally in the dog: Ligation with complete obstruction of the common bile duct^{2,3,4} results in extremely high serum phosphatase levels; hepatitis produced by hepatotoxic drugs^{4,5,6} or by Leptospiral inoculation⁴ causes increases in serum phosphatase generally considerably less than those observed after common duct ligation, though hyperbilirubinemia may be as marked or more marked.;

The dissociation of serum phosphatase and serum bilirubin levels observed clinically with incomplete obstruction of the biliary tract has been little studied experimentally but was reproduced by Freeman, Chen and Ivy⁴ in 2 dogs following ligation of hepatic ducts draining approximately ½ of the liver. Though jaundice did not develop, the serum phosphatase rose to 25.8 and 37.9 Bodansky units per 100 cc, respectively; in the one instance followed, there was a spontaneous, gradual fall to almost normal levels after 2 months. Confirmatory data are recorded here together with such additional studies on the urine as bear upon the interpretation of the blood changes.

Methods. In 4 dogs following nembutal anesthesia, the hepatic ducts were identified by aspiration of bile and a variable number (draining 1/5, 1/4 or 1/2 of the liver) were tied off with silk. Periodic postoperative examinations were made of the serum for phosphatase (Bodansky method), total cholesterol (Bloor method) and bilirubin, and of the urine for "alkaline" phosphatase (King

¹ For references and additional data see Gutman, A. B., Olson, K. B., Gutman, E. B., and Flood, C. A., J. Clin. Invest., 1940, 19, 129.

² Bodansky, A., and Jaffe, H. L., Proc. Soc. Exp. Biol. AND Med., 1934, 31, 1179.

³ Armstrong, A. R., King, E. J., and Harris, R. I., Canad. M. A. J., 1934, 31, 14.

⁴ Freeman, S., Chen, Y. P., and Ivy, A. C., J. Biol. Chem., 1938, 124, 79.

⁵ Armstrong, A. R., and King, E. J., Canad. M. A. J., 1935, 32, 379.

⁶ Bodansky, A., Enzymolog., 1937, 3, 258.

[†] An interesting exception is m-toluylenediamine jaundice in dogs, which is associated with very high scrum phosphatase and cholesterol values. This type of jaundice, however, appears to be partly hemolytic, partly due to stasis of bile in the finer biliary radicles rather than to parenchymal injury. A somewhat analogous condition, with high scrum phosphatase, is seen clinically in certain drug-hypersensitive cases of post-aisphenamine jaundice, in which liver biopsics show obstruction of the intrahepatic biliary tract.

⁷ Naunyn, B., Mitt. Grenzgeb. Med. u. Chir., 1919, 31, 537.

⁸ Hanger, F. M., and Gutman, A. B., J. A. M. A., in press.

obstruction is not inconsistent with the "phosphatase retention" theory. As to whether the increased serum phosphatase is of hepatic or osseous origin, our results do not exclude the one or the other possibility since occlusion of the excretory biliary channels would tend to cause retention of bile constituents of both hepatic and extra-hepatic origin.

Summary. Ligation of hepatic ducts in 4 dogs resulted in increased phosphatase but not bilirubin in the serum, increased bilirubin but not phosphatase in the urine. The dissociation in the blood is thought to be due largely to differential renal excretion and to be consistent with the "phosphatase retention" theory.

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Breast Cancer Produced in Male Mice of the C57 (Black)
Strain of Little.

GRAY H. TWOMBLY. (Introduced by C. P. Rhoads.)

From Memorial Hospital, New York City.

Bittner¹ has shown that hybrids from a cross between a mouse from a strain having a high incidence of mammary cancer and one from a low tumor strain have a high or low incidence of breast cancer depending on what type of mother they nurse. If the nursing mother comes from the strain having the high incidence of spontaneous tumors, a large percentage of the female hybrids will develop mammary cancer. If they are nursed, on the other hand, by a female from the low tumor strain very few will develop breast cancer. Female mice from a high cancer strain nursed by their own mothers have a high incidence of mammary cancer while if they are foster-nursed by a mouse from a low tumor strain the chance that they will develop breast cancer will be materially reduced.

An attempt was made to confirm this observation on a different strain of animals. Mice of the RIII (Paris) strain of Dobrovolskaia Zavadskaia were given to a female of the C57 (black) strain of Little to nurse while the young of the latter were given to the RIII mother. The RIII females have an incidence of spontaneous mammary cancer of 70% in virgin females. The incidence in C57 black females is less than 1%. The incidence of breast

¹ Bittner, J. J., Am. J. Ca., 1939, 35, 90.

bile retention would seem to be the differential excretion of bilirubin and phosphatase in the urine.

In man with incomplete obstruction of the biliary tract and therefore only moderate retention of bile, urinary excretion of bile pigments may increase sufficiently to maintain the patient virtually free of jaundice. The human kidney is impermeable to serum phosphatase, however, and this difference in available excretory channels appears to be partly responsible for the observed dissociation of serum phosphatase and bilirubin levels. In the dog, the blood and urine studies recorded here suggest that the same factors are responsible for the analogous dissociation in serum levels. By varying the number of hepatic ducts tied off, a positive correlation between the serum phosphatase level and the degree of obstruction could be demonstrated. The serum phosphatase values in our dogs are relatively high as compared with man but this corresponds with the extraordinarily high values in dogs with complete biliary obstruction; the serum bilirubin levels are relatively low, due to the greater clearance of the dog kidney. In the cat, on the other hand, "alkaline" phosphatase as well as bilirubin appears in the urine and complete obstruction of the common bile duct results in comparatively little rise in either serum phosphatase or bilirubin.10

Factors other than differential renal excretion may contribute to the dissociation of serum phosphatase and bilirubin levels following bile retention since their level in the serum represents a quite complex dynamic equilibrium between the rate of secretion into the blood stream and the rate of excretion (by one or more channels), metabolism or deposition in the tissues. Though not open to direct measurement, the important influence of these latter factors on the serum phosphatase level is indicated by the wide range in serum phosphatase values in man with complete common duct obstruction. 1½

We conclude that the occurrence of increased serum phosphatase activity with little or no jaundice following incomplete biliary tract

⁹ Flood, C. A., Gutman, E. B., and Gutman, A. B., Am. J. Physiol., 1937, 120, 696.

¹⁰ Cantarow, A., Stewart, H. L., and McCool, S. G., Proc. Soc. Exp. Biol. and Med., 1936, 35, 87.

that in the latter case there is selective impairment of different liver functions by various hepatotoxic drugs.

¹¹ Bodansky, A., Proc. Soc. Exp. Biol. and Med., 1939, 42, 800.

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¹ Bittner, J. J., Am. J. Ca., 1939, 35, 90.

cancer occurring in these 2 groups of animals appears to confirm Bittner's observations but the number of animals is as yet not very statistically significant and consequently will be reported later in another place.

Since litters were shifted within less than 12 hours of birth when the sex was often hard to determine, many males which had been foster-nursed by mothers of the other strain became available. It has been proved² that male mice of a strain showing a high incidence of spontaneous breast cancer in the females will develop cancer more quickly than their littermate sisters if a crystal of oestrone weighing 0.1 mg is implanted in them subcutaneously on the tenth day after birth. Because this procedure was easy and the mice were available the C57 males nursed by RIII females were so treated.

Many animals have been used, a fair proportion of which have died of hyperestrinism before they were old enough to show tumors. Some are still too young for tumors to have developed. Nine males so treated have had spontaneous mammary cancer, 8 of which have been proved histologically. The ninth died in the night and was eaten by his cage-mates before morning. The earliest tumor appeared at 7½ months after birth in a mouse implanted with 0.18 mg of crystalline oestrone. The oldest mouse to develop a tumor did so 11 months after birth after a dose of 0.09 mg of oestrone. The average age at which tumors appeared was 9 months. So far 27 animals have lived 9 months or longer or have developed tumors before the ninth month. Fourteen of these animals are still alive. In this small series then we may say that by foster nursing C57 black male mice with RIII (Paris) mothers and implanting them at 10 days of age with a crystal of oestrone weighing 0.07 to 0.18 mg, 9 out of 27, or 33%, have developed breast cancer.

This observation assumes greater importance when one considers that mammary cancer has not been observed previously in C57 black male mice. Haagensen in a personal communication reports the treatment of 107 males with maximal doses of oestrone benzoate in oil twice a week from 10 days of age to death without producing a single tumor. Gardner has had 3 tumors in 250 mice treated. It would seem that breast cancer in the animals reported in the present communication is due not only to hormonal stimulation of the male breast tissue but to some agent or influence transmitted in the mother's milk other than oestrogenic substances.

² Twombly, G. H., Proc. Soc. Exp. Biol. and Med., 1939, 40, 430.

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Production of Gastric and Duodenal Ulcers in the Cat by Intramuscular Implantation of Histamine.*

STEWART H. WALPOLE, RICHARD L. VARCO, CHARLES F. CODE AND OWEN H. WANGENSTEEN.

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The finding of Code and Varco¹ that prolonged stimulation of gastric secretion could be obtained by the injection of a histamine beeswax mixture into dogs offered a means of testing whether endogenous gastric secretion could produce ulcers of the stomach or duodenum. Cats were chosen for this investigation, because it had been found in a recent study in this laboratory that these animals developed erosions and ulcerations of the stomach and duodenum quite readily when 0.4% hydrochloric acid was instilled daily into the stomach through a fistula (Walpole). It seemed possible that a profitable comparison might be made between ulcer production in response to endogenous and exogenous acid.

Experimental Procedure. The problem was approached by first studying the effects of single injections of the histamine beeswax mixture on gastric secretion and then observing the effects produced by chronic histamine administration. The effect of the histamine beeswax mixture upon gastric secretion was studied in 3 cats with a standard type of gastrostomy.

Controls. Control observations were made with 2 of these animals some days prior to the injections of histamine. The continuous secretion of the fasting stomach was collected every 8 hours for 24 hours. Several days later each cat was given an intramuscular injection of plain beeswax mixture equivalent in amount to that in a dose of the histamine beeswax mixture and the continuous gastric secretion was collected every 8 hours for 24 hours. In addition, in 3 control cats beeswax alone was implanted daily intramuscularly over periods varying from 8 to 23 days. In each instance, the cat gained weight and its general health appeared

^{*}This research was supported by grants from the Committee on Scientific Research of the American Medical Association (grants 526 and 556) and by a grant from the Graduate School of the University of Minnesota; also by a grant for technical assistance by the Work Projects Administration, Official Project No. 665-71-3-69, Sub-project 258.

¹ Code, C. F., and Varco, R. L., PROC. Soc. Exp. Biol. AND Med., 1940, 44, 475.

satisfactory. One of these cats was killed and subjected to a careful necropsy. There was no ulcer. The other cats continued in good health. During the period of this study, 13 cats kept in the laboratory for other purposes have been subjected to necropsy. No ulcers were found. The cat has been used periodically as an experimental animal in the surgical laboratory over a period of years. No spontaneous ulcers have been observed.

Method. The acidity of the gastric juice was determined by colorimetric titration. In both groups the quantity of secretion obtained and the maximum free acidity produced were considerably lower than that following stimulation with histamine in beeswax. The maximum free acidity of the gastric juice following administration of plain beeswax was 48 clinical units, and of 6 samples collected 3 had no free acid. The maximum free acidity attained by the fasting stomachs was 66 clinical units, and of 6 samples collected 3 contained no free acid. The experiments indicate that plain beeswax in the dose given had no effect upon gastric secretion.

As a routine, the dose of histamine used in the beeswax mixture was 20 mg of the free base. This was injected in divided portions into the muscles of the back. In the cats with gastric fistulae, following administration of this dose, either fractional samples were collected or the entire continuous secretion was taken at hourly intervals for 24 hours. Food was withheld for 24 hours preceding injection. As in the dog (Code and Varco) there was a constant copious secretion of gastric juice. After a short lag during the first hour, the free acid rose to values of more than 100 clinical units but usually fell later to a somewhat lower range. In only 2 samples of 108 collected was free acid absent.

To determine the effect of this prolonged abundant flow of gastric juice with high free acidity upon the stomach and duodenum, 7 healthy cats weighing 4 to 8 lb were given each a daily dose of 20 mg of histamine in beeswax injected intramuscularly. The animals were fed each morning and the injections made several hours later. Adequate fluids were supplied by subcutaneous administration of normal saline solution. The animals were sacrificed when they appeared obviously ill, or refused food on 2 successive days, or after blood was noted in several specimens of vomitus.

Histamine in beeswax frequently produced no obvious reaction, but occasionally the injection was followed immediately by a typical chain of symptoms. This consisted of restlessness, increased respirations, and vomiting associated often with salivation and passage of a loose stool. The occurrence of these symptoms seemed to

depend more upon the batch of histamine mixture used than upon the individual animals. Recently, with increased experience in preparation of the material, reactions have been less frequent. In all animals, however, vomiting occurred some time during the course of injections and when not associated with the immediate reaction took place several hours after the injection. The vomitus usually contained free acid.

These 7 cats receiving the histamine beeswax mixture were sacrificed at periods ranging from 3 to 25 days after beginning the injections, the total amount of histamine given ranging from 60 to 480 mg. At necropsy there were erosions or acute ulcers of the stomach or duodenum, or both, in all animals. Two cats had lesions of the duodenum only, 2 had lesions of the stomach only, and 3 had lesions of both stomach and duodenum. In 3 animals there were persorated ulcers, two in the duodenum and one in the stomach. Gastric lesions were limited to the antrum. It seemed obvious that the animals had been sacrificed at various stages of ulcer formation.

Comment. Histamine in beeswax prepared according to the method of Code stimulated a sustained copious flow of gastric juice containing free acid when injected intramuscularly into cats. Repeated injections of histamine in saline solution have been reported as failing to produce ulceration in the gastro-intestinal tract in the dog² and also in the cat.³ Repeated single daily doses of histamine in beeswax in this study were effective in the cat in producing erosions and all stages of ulceration including acute perforation. one dog tested, ulceration occurred in the duodenum.† These findings suggest the importance of the gradual liberation of histamine from beeswax in maintaining a constant and fairly uniform stimulation of gastric secretion, as opposed to the intermittent stimulation afforded by periodic injections of histamine in watery solution.

² Orndorff, J. R., Bergh, George S., and Ivy, A. C., Surg. Gynec. Obstet., 1935, 61, 162.

³ Heinlein, H., and Kastrup, H., Z. f. d. ges. exp. Med., 1938, 102, 517.

t Since this paper was written intramuscular implantation of histamine has been done in an additional eat and in another dog. Both animals were killed and autopsied when it was apparent that they were ill. The cat had a large ulcer in the fundus 12 days after the administration of histamine was begun. The dog had a small ulcer .5 em in diameter in the first portion of the duodenum 4 days after histamine administration was begun.

11548

Relationship Between Insulin Dosage, Duration and Degree of Hypoglycemia and Production of Brain Damage.*†

DAVID B. TYLER AND EUGENE ZISKIND. (Introduced by D. R. Drury.)

From the Department of Medicine, School of Medicine, University of Southern California.

This report deals with the relation of insulin dosage, duration and degree of the ensuing hypoglycenia, and the resulting brain damage produced. Observations of a paradoxical reaction with insulin are also recorded.

It is well established from the work of Scott and Dotti,1 Zucker and Berg,2 and many others, that from 20 to 60 minutes after the injection of insulin, the blood sugar reaches its lowest level and remains there with minor fluctuations for a period depending upon the amount of insulin given. With insulin doses such as we used, of from 10 to 20 units per kilo of bodyweight, the hypoglycemia persists from 10 to more than 24 hours. However, independent of the hypoglycemia certain clinical symptoms occur which indicate a progressive loss of function, in a phylogenetic order, from the higher cortical areas to the lower or medullary centers.3-6 In this connection, Frostig' described 4 stages of hypoglycemia in man after large doses of insulin, based on impairment of the function of (1) cerebral cortex, (2) basal ganglia and thalamus, (3) midbrain, and (4) medulla. In our previous report, certain interesting data became apparent bearing on the problem of insulin dosage in relation to brain damage.

The method used has been described in a previous report.⁷ Briefly stated, doses of insulin were given subcutaneously to cats fasted 18 hours and not previously treated with insulin. Complete

^{*} Insulin was kindly furnished by Eli Lilly & Co.

t We are indebted to Dr. C. H. Thienes of the Department of Pharmaeology for the use of laboratory space and many helpful suggestions.

¹ Scott, E. L., and Dotti, L. B., Arch. Int. Mcd., 1932, 50, 511.

² Zucker, T. F., and Berg, B. N., Am. J. Physiol., 1937, 119, 531.

³ Augyal, L. V., Z. Neur. and Psychiat., 1937, 157, 35.

⁴ Frostig, J. P., Arch. Neur. and Psychiat., 1938, 39, 219.

⁵ Himwich, H. E., Frostig, J. P., Fazekas, J. F., and Hadidian, Z., Am. J. Psychiat., 1939, 96, 371.

⁶ Ziskind, E., and Tyler, D. B., in preparation.

⁷ Ziskind, E., and Tyler, D. B., PROC. Soc. EXP. BIOL. AND MED., 1940, 43, 734.

quarter-hour notations of the neurological state of the animal were made. When the animal showed signs of critical medullary decompensation, small amounts of glucose (100-200 mg) were given intraperitoneally as needed, in order to restore the circulation, pulse and respiration, but still maintain a severe hypoglycemia. The hypoglycemia was terminated after 9 to 20 hours with glucose. We considered those animals had brain damage which showed the irreversible clinical symptoms of "decortication" and "decerebration" described elsewhere. In this report when we describe a "stage" of hypoglycemia such as the "medullary stage," we refer to the neurological symptoms at that time.

1. The Relation Between Insulin Dose and Incidence of Brain Damage. Column III of Table I shows that the greater the dosage the greater the incidence of residual brain damage. In animals receiving 10, 15 and 20 units of insulin per kilo, the incidence of brain damage was respectively 30%, 33%, and 62%. These results cannot be correlated with the duration of hypoglycemia or the period of coma (columns IV and V). These findings correspond to those previously reported by Yannet.⁸

TABLE I.

I			IV	V
Insulin			Hr of	Hr
dose			hypoglycemia	coma
20 u./kg 15 u./kg 10 u./kg Less than 5 u./kg	4.16 (20) 3.67 (43) 3.28 (20) 2.24 (12)	62% (13) 33% (33) 30% (20)	11 13 7.5	7 9 4.5

Number in parenthesis indicates number of animals.

- 2. The Relation of Duration of Medullary Stage to Brain Damage. Residual brain damage did not occur in our series irrespective of the dose of insulin unless the animal was in the "medullary stage" for not less than 100 minutes. This medullary stage (Stage IV of Frostig) is characterized by pin point pupils, bradycardia, respiratory irregularities especially Cheynes-Stokes respiration, flaccidity, and finally symptoms of circulatory collapse. However, the time that the animal must be kept in this stage to produce brain damage varies indirectly with the body temperature of the animal. These findings do not lend support to the thesis that insulin per se is toxic to the brain cell.
 - 3. Paradoxical Relation of Increasing Dose of Insulin and Disappearance of Cerebral Functions. The larger the dose of insulin

⁹ Yannet, H., Arch. Neur. and Psychiat., 1939, 42, 395

(within the limits of our experiments) the longer the latent period before cortical functions disappear, as gauged by the loss of consciousness and the time of appearance of the first myoclonic jerks. (Column II of table.) The average time of appearance of the first myoclonic jerks in cats receiving 5 u/kg or less was 2.24 hours, 10 u/kg 3.28 hours, 15 u/kg 3.67 hours, and 20 u/kg 4.16 hours. We are unable to suggest the reason for this reaction.

Conclusions. 1. The larger the dose of insulin the greater the incidence of brain damage. However, irrespective of the insulin dose, brain damage did not occur in our animals unless they were kept in the "medullary stage" of hypoglycemia for at least 100 minutes. 2. In cats, not previously treated with insulin, the larger the dose the longer time it took for the appearance of neurologic signs of hypoglycemia.

11549 P

Elimination of Metrazol.

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Pharmacological and clinical evidence points to the fact that metrazol is very rapidly detoxicated in the body. The intravenous administration of a convulsive dose produces typical clonic convulsions from which the animal rapidly recovers.

There is a possibility that the metrazol might be excreted by the kidneys, and so our first step consisted in eliminating this possibility. Chemical analysis of the urine of cats receiving convulsive doses of metrazol showed none of the drug to be present in the urine. Bilaterally nephrectomized cats showed the same reaction to a convulsive dose of metrazol as they did before the performance of the nephrectomy. Hinsberg has shown that practically no metrazol is excreted by the intestinal route. It therefore seems logical that metrazol is not excreted but is detoxified.

The liver has generally been assumed to be the locale for drug detoxication. We therefore administered phosphorus to cats. Cats treated in this way died from the administration of a dose of metrazol which formerly produced only slight convulsions. The role of the liver was further tested by a comparison of the dose required to

produce convulsions when the drug was infused into the marginal ear vein or the portal vein of rabbits. In all cases a larger amount was required to produce convulsions when administered by the portal route. This evidence seems to establish the fact that metrazol is detoxified rather than excreted and that the liver plays an important rôle in the detoxication process.

11550

Coronary Occlusion. II. Efficacy of Papaverine Hydrochloride in Treatment of Experimental Cardiac Infarction.

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From the Department of Pharmacology, College of Physicians and Surgeons,

Columbia University, New York City.

Papaverine has been suggested repeatedly as a drug of therapeutic value in the treatment of coronary artery disease. This recommendation has been based on the thesis that the vaso-dilator action of papaverine would increase local blood flow. The anginal attack is thus relieved, or the ultimate size of the myocardial scar is minimized as a result of the improved nutrition.

Allen and MacLean⁴ stated that the pain in peripheral arterial embolization is due not only to the presence of the embolus, but also to the attendant widespread reflex vascular spasm. The extensive ischemia that they observed was more marked than could be accounted for by the occlusion of the main vessel alone. In arterial embolization papaverine is said to have effects analogous to sympathectomy, *i.e.*—relaxation of the vaso-spasm. The use of papaverine has been suggested also in cerebral, pulmonary and mesenteric occlusion.^{3,4} Mulinos, Shulman and Mufson⁵ found that vaso-spasm of Reynaud's disease was relieved by large doses of papaverine hydrochloride intravenously, doses which did not lower the blood pressure but which resulted in a moderate acceleration of the heart rate.⁶ Gruber and Robinson⁷ noted that papaverine in small

¹ Semler, R., Med. Welt., 1928, 2, 335.

² Neu, J., Therap. d. Gegenw., 1927, 68, 564.

³ De Takats, G., J. A. M. A., 1936, 106, 1003.

⁴ Allen, E. V., and MacLean, A. R., Proc. Staff Meet. Mayo Clinic, 1935, 10, 216.

Mulinos, M. G., Shulman, I., and Mufson, I., Am. J. Med. Sci., 1939, 197, 793.
 Mulinos, M. G., and Shulman, I., J. Pharm. Exp. Therap., 1939, 66, 27.

⁷ Gruber, C. M., and Robiuson, P. I., J. Pharm. Exp. Therap., 1929, 37, 429.

doses caused an increase in the height of the contractions in the isolated perfused terrapin heart. Macht⁸ observed that small doses caused slowed, more powerful contractions of the isolated frog's heart.

Coronary occlusion, whether due to embolus, spasm, or ligation, results in a central area of almost complete ischemia which is surrounded by a halo of myocardial edema and reflexly spastic vessels. These peri-infarctial coronary vessels are of importance because they supply blood to the adjacent myocardium and also because they are potential anastomotic connections with the infarcted area (Wearn⁹). It is to be expected, therefore, that vasodilators which decrease the vaso-spasm should prove beneficial by increasing the anastomotic circulation, and also by lessening the peri-infarctial edema, thus diminishing the ultimate size of the infarct. No experimental work has been done in support of the contention that papaverine exerts such a beneficial effect on the course of occlusive coronary disease. The present study attempts to determine the effect of papaverine upon the size of an experimentally produced myocardial infarct. Gold, Travell and Modell10 and Fowler, Hurevitz and Smith11 have made similar studies employing aminophylline as the vasodilator.

Method. Using the method described previously, 12 the left branch of the left anterior descending coronary artery was ligated aseptically in 22 cats under sodium pentobarbital anesthesia. An attempt was made to tie the vessel at the same point in each animal. Standard 3 lead electrocardiograms, white and differential blood counts, and determinations of the sedimentation rate were made pre-operatively, and at intervals in the post-operative life of the cats. Following the ligation alternate cats received 5 mg per kilo of papaverine hydrochloride (1 cc = 30 mg)* intramuscularly twice a day for a period of 2 weeks (Sundays excepted). The interval cats were kept as controls and were treated analogously except for the injections. All the cats remained in good condition until they were sacrificed.

⁸ Macht, D. I., Arch. Int. Med., 1916, 17, 786.

⁹ Wearn, J. T., Harvey Lecture, 1940.

¹⁰ Gold, H., Travell, J., and Modell, W., Am. Ht. J., 1937, 14, 248.

¹¹ Fowler, W. M., Hurevitz, H. M., and Smith, F. M., Arch. Int. Med., 1935, 56, 1242.

¹² Scott, W., Leslie, A., and Mulinos, M. G., Am. Ht. J., 1940, 19, 719.

^{*} Generously contributed by Eli Lilly and Company.

TABLE I.
Infarct Size and Its Relation to Age of Infarct and Administration of Papaverine.

	Ţ	reated cats		Unt	reated cats
Cat No.		Infarct arca, cm ²	Cat No.	Days	Infarct area,
18	7	0.88	9	24	1.33
29	7	3.19	5	27	1.59
25	14	3.76	3	28	1.62
16	15	2.24	12	29	1.22
13	20	1.76	27	30	0.55
26	20	1.52	10	30	2.06
6	21	2.91	11	30	1.30
14	21	0.80	7	33	1.07
4	29	3.64	8	44	2.15
19	101	0.39	9	47	1.58
			10	50	4.40
			21	56	None seen
~~		_			_
10	Avg 25.5	Avg 2.11 $_{G} = 0.363$	12	Avg 35.7	Avg 1.57 $\sigma = 0.324$

From 6 to 102 days after operation, the cats were killed by the intravenous injection of chloroform. The hearts were excised, the infarct delineated with ink and a contact tracing of each infarct was made. The areas of the tracings, shown in Table I, were determined by means of a planimeter. There is no obvious correlation between the size of the infarct and its age.

Results. The treated cats experienced some immediate pain from the injection of the papaverine hydrochloride, which has a pH of 2, but showed no other untoward effects. There was no depression, vomiting, or loss of appetite.

As shown in the Table, the area of infarct averaged 34% larger in the papaverine treated cats than in the uninjected controls. This difference falls well within the average deviation of either series and is consequently considered as accidental. In large part the size of the infarct depends in the different cats upon the anatomical distribution of the coronary vessel which is ligated at the operation. The differences in the area of the infarcts are shown in the Table as a scatter of from 0.39 sq cm to 3.76 sq cm for the papaverine treated cats and of from zero to 4.40 sq cm for the control animals. A larger series might be desirable. However, more data would merely result in lowering of the average deviation of infarct size, without throwing additional light upon the effect of the drug. Our figures indicate that infarct size cannot be used as a criterion of coronary vasodilator action and therefore it is felt that the coronary vasotropic effect of papaverine must be studied by other methods as well. Papaverine is being compared by us with nitroglycerine

and theophylline for its effects upon the electrocardiogram with and without induced anoxemia. The length of time necessary for the recovery of the electrocardiogram and for the return of the white blood cell count and sedimentation time to normal was roughly proportional to the size of the infarct, and independent of the administration of the papaverine. From our failure to demonstrate any reduction in the size of the infarct after coronary ligation, it cannot be concluded that papaverine hydrochloride has no place in the clinical treatment of coronary occlusion or of angina pectoris.

Conclusion. The daily injection of papaverine hydrochloride (5 mg per kilo) into cats for 2 weeks did not alter significantly the size of the infarct resulting from the ligation of the left branch of the left anterior descending coronary artery.

It is suggested that because of the greatly variable amount of cardiac tissue involved in each ligation this method of study is too crude to detect any "clinical" improvement that the drug may have exerted.

11551

Anaphylactic Shock and Susceptibility to Histamine Poisoning in the Cotton Rat Sigmodon hispidus littoralis.*

BEATRICE CARRIER SEEGAL

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.

The Eastern cotton rat Sigmodon hispidus hispidus and the Florida cotton rat Sigmodon hispidus littoralis have come into prominence as laboratory animals because of their reported susceptibility to the virus of poliomyelitis. The cotton rat is a small rodent and apparently it is capable of adaptation to laboratory life. It is therefore of interest to explore its usefulness for other experimental purposes. Reports on a natural trypanosome infection of the Florida cotton rat, on the susceptibility of this animal to diph-

^{*}This work was supported by a grant from the Philip Hanson Hiss, Jr., Memorial Fund.

¹ Armstrong, C., Pub. Health Rep., 1939, 34, 1719.

² Jungeblut, C. W., and Sanders, M., Proc. Soc. Exp. Biol. And Med., 1940, 44, 375.

³ Culbertson, J. T., J. Parasit., in press.

theric toxin,⁴ and to infection with the tubercle bacillus⁵ have been made. The present communication describes attempts to produce anaphylactic shock in the Florida cotton rat. In addition to testing for hypersensitivity, the serums of some of the animals, obtained after sensitization, were tested for precipitin content. The animals subsequently were subjected to intravenous injections of histamine.

Sheep serum as an anaphylactogen: One or 2 sensitizing injections of sheep serum were given intravenously to 8 cotton rats. The total amount used for sensitization ranged from 0.05 to 0.6 cc. After an incubation period, varying from 17 to 29 days, the animals were reinjected intravenously with 0.25 to 0.50 cc of sheep serum. In no case was any reaction obtained following the shocking injection of antigen. Six of these animals were retested for sensitivity 17 days later by the intravenous injection of 0.3 to 0.5 cc of sheep serum. The animals were again equally refractory to shock.

Five of the animals were bled 19 or 25 days after the last shocking injection of antigen, and their serums tested for precipitins to sheep serum. In 3 of the serums no precipitins could be demonstrated; in 1 serum there was a trace with antigen diluted 1:20, while the last serum reacted to give a definite precipitate with antigen diluted 1:10, 1:40 and 1:160 and a trace with antigen diluted 1:640.

Whole egg white as an anaphylactogen: Two sensitizing injections of 0.5 cc of 25% whole egg white were given 3 days apart to 9 cotton rats. The first injection was given intravenously, the second intraperitoneally. Five of the 9 animals had previously been used in the sheep serum experiments. After a 21-day incubation period, all 9 animals received 0.5 cc of the same egg white solution intravenously. Seven of the cotton rats failed to show any reaction. One animal was listless for about 20 minutes, sitting in one corner of the cage and responding only sluggishly to prodding. The last animal showed a greatly increased rate of respiration for about 5 minutes after injection. It then appeared normal for about 15 minutes when it was observed that the animal had become limp and could not be aroused to activity. Respirations were slow but not labored. The animal lay on its side, became progressively weaker with slower respiration during another 15 minutes and then stopped breathing, 35 minutes after the injection of egg white. Autopsy revealed no gross lesions in the heart or lungs. Each pleural cavity contained a drop of blood or bloody fluid, and there was a small amount of serosanguinous fluid in the peritoneal cavity.

⁴ Jungeblut, C. W., Proc. Soc. Exp. Biol. and Med., 1940, 43, 479.

⁵ Steinbach, M. M., and Duca, C. J., Proc. Soc. Exp. Biol. and Med., 1940, 44, 288.

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mucosa of the stomach had one area of hemorrhage 4 mm in diameter and one of the Peyer's patches of the small intestine was studded with petechial hemorrhages. The type of death and the paucity of findings at autopsy suggested the picture of anaphylaxis in the rat.

Four of the 9 animals sensitized to egg white were bled from the heart 4 hours before the intravenous shocking injection of antigen. Serums from these bloods were tested for precipitins. Three of the serums contained no demonstrable precipitins, while the fourth showed only a trace of precipitate when tested with antigen diluted 1:20 and 1:100.

Histamine shock: Eight cotton rats were tested for their susceptibility to histamine (ergamine acid phosphate, Burroughs Wellcome & Co.). The drug was dissolved in 0.85% NaCl so that each cubic centimeter contained 1 mg. The cotton rats, weighing between 130 and 175 g, were injected intravenously with 0.4 to 1.5 mg. The minimal lethal dose proved to be roughly 1 mg of histamine for a 130 g cotton rat, or approximately 0.8 mg per 100 g. This amount killed 4 out of 5 animals, whereas 0.6 mg per 100 g respiratory rate in the 3 animals tested. There was considerable of cotton rat failed to produce more than transitory increase in individual variation in the mode of death. In 1 animal (weight 132 g) death occurred in 3 minutes following injection of 1 mg of histamine. It was characterized by frothing at the nose and labored respiration and by tonic and clonic convulsions and opisthotonos. At autopsy the lungs were slightly distended and hemorrhagic. A second animal (weight 175 g) died one hour following the intravenous injection of 1.5 mg. This animal did not show discharge of froth from the nose and the respirations, although rapid, were not labored. It lay on its side, prostrated, for 45 minutes before death. On autopsy the lungs appeared normal. There were many petechial hemorrhages in the Peyer's patches and also scattered through the small intestine. A third rat, also 130 g in weight, died in 3 hours following the injection of 1 mg. In this case, difficulty of respiration was also absent. Periods of complete prostration alternated with periods of violent tonic and clonic convulsions. The only findings at autopsy were petechial hemorrhages in Peyer's patches. A fourth cotton rat, which had also been given 1 mg of histamine, showed only some increase in the rate of respiration for 30 minutes following injection, but looked sick and listless the following day and died 24 hours later without showing any lesions at autopsy.

The potency of the histamine was tested in 3 guinea pigs weighing 700 g. Two of these animals died acutely following the injection of 0.4 mg while the third survived 0.3 mg.

Conclusions: The cotton rat was found relatively refractory to anaphylactic shock. In this respect it resembles the ordinary laboratory rat. Low titered precipitins occurred in 3 of the 9 serums tested after sensitization. The minimal lethal dose of histamine intravenously was approximately 0.8 mg per 100 g for the cotton rat. This is 15 times the quantity required to kill a guinea pig, but is 100 times less than that which has been reported lethal for the rat.

11552

Relation Between Volume of Vehicle and Chick Comb Response to Androsterone.*

EMANUEL KLEMPNER, FRANKLIN HOLLANDER AND ROBERT T. FRANK.

From the Laboratories of the Mount Sinai Hospital, New York.

It was suggested previously (Frank, Klempner and Hollander¹) that, in the use of sesame oil as a vehicle in our bioassay method for androgens, a reduction in the volume of vehicle from 0.1 cc to 0.05 cc was one of the factors which contributed to the improvement in response. Subsequently, the possibility presented itself that a further reduction in this volume might effect further improvement, as manifested by increased comb growth for a given dose of androgen. Accordingly, we have investigated the response elicited by the application of various dosages of androsterone in 0.05 cc and in 0.02 cc† of oil, applied daily, in paired experiments run simultaneously. In all other respects, the experimental conditions were exactly the same as in our last report (loc. cit.).

The results of such paired experiments are summarized in Table

^{*}This investigation was supported in part by a grant from the Friedsam Foundation.

¹ Frank, R. T., Klempner, E., and Hollander, F., Proc. Soc. Exp. Biol. And Med., 1938, 38, 853.

t In order to facilitate the application of these small volumes, as well as to control accuracy of delivery, a simple mechanical device was attached to the syringes used. This device, constructed by Mr. Vondrak, chief technician of laboratorics, Mount Sinai Hospital, will be described elsewhere.

mucosa of the stomach had one area of hemorrhage 4 mm in diameter and one of the Peyer's patches of the small intestine was studded with petechial hemorrhages. The type of death and the paucity of findings at autopsy suggested the picture of anaphylaxis in the rat.

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ment with the larger volume. Each of these response values has likewise been calculated as per cent of the corresponding control value and is given in column 8. The improvement in response resulting from reduction in volume of sesame oil (IR, column 9) is therefore measured by the difference of these paired percentage values. These "improvement" values are uniformly positive, corresponding to the greater response with 0.02 cc of oil than with 0.05 cc. They vary in magnitude from 43% to 108% of the corresponding control comb weight. The mean improvement is 67.9%, with a standard deviation of 5.4, and is based on a total of 710 chicks: 277 treated with the larger volume of oil, 278 with the smaller volume, and 155 untreated controls. A second group of experiments, identical with the foregoing but restricted to the dosage range 1-9 y inclusive, has also been performed. This series employed a total of 308 chicks, of which 238 were treated and 70 were controls. The results were similar to those in the foregoing series.

It may be concluded, therefore, that this further reduction in volume of vehicle effects a further increase in the comb growth response of baby chicks. The only explanation which we can offer at the present time for this improvement is that the smaller the volume, the greater is the proportion of androgen solution utilized on the comb surface instead of being diverted to adjacent, less-sensitive head areas.

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

11553

Improvement in Chick Comb Response to Androsterone Obtained with Alcohol as Vehicle.*

EMANUEL KLEMPNER, ROBERT T. FRANK AND FRANKLIN HOL-LANDER.

From the Laboratories of the Mount Sinai Hospital, New York

In our studies of the bioassay method for androgens, utilizing the comb of the baby chick, it has appeared that further improve-

^{*} This investigation was supported in part by a grant from the Friedsam Foundation.

TABLE I.

Effect of Further Reduction in Volume of Vehicle (Sesame Oil) in the Bioassay of Androgens by the Chick Comb Method.

		Vol.	Response to androgen application Improve- (comb wt corrected ment					
I	Androgen	of	No.	Treated	Control		ntrols)	in
	dosage	oiI	of	chicks	chicks			response
No.	(γ)	(cc)	chicks	W (mg)	W _c (mg)	(W-W _c)	% of W	(IR)2
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
27	10	.05	25	34.0	18.5	15.5	84	+52
		.02	25	43.6	(28)*	25.1	136	
28	10	.05	30	31.1	17.5	13.6	78	+63
		.02	30	42.2	(26)	24.7	141	
29	10	.05	30	31.0	19.3	11.7	61	+89
		.02	30	48.3	(23)	29.0	150	
30	10	.05	29	35.6	17.7	17.9	101	+75
		.02	29	48.8	(31)	31.1	176	
26	12	.05	15	43.1	23.0	20.1	87	+56
		.02	15	55. 9	(21)	32.9	143	
26	15	.05	14	45.7	23.0	22.7	99	+63
		.02	15	60.2	(21)	37.2	162	
28	15	.05	30	39.2	17.5	21.7	124	+66
		.02	30	50.8	(26)	33.3	190	
29	15	.05	30	38.1	19.3	18.8	97	+ -79
		.02	30	53.3	(23)	34.0	176	
25	30	.05	25	59.2	16.0	43,2	270	+-5 3
		.02	25	67.6	(26)	51.6	323	
27	30	.05	24	49.1	18.5	30.6	165	+108
		.02	24	69.0	(28)	50.5	273	1.40
25	50	.05	25	71.0	16.0	55.0	344	+43
		.02	25	77.9	(26)	61.9	387	ar and
							mean :	= 67.9%
							σ _M =	= ±5.4%

*Figures in parentheses No. of chicks.
$$\frac{W-W_c}{W_c} \times 100$$
1 Response as % of $W_c = \frac{W-W_c}{W_c} \times 100$
2 Improvement (IR) as % of $W_c = \frac{W_{.02}-W_{.05}}{W_c} \times 100$

I, using mean comb weight for each group of chicks, regardless of sex, which had been treated in the same way. The dosages ranged from 10 to 50 γ. The response to treatment in any one experiment was measured by the increase in comb growth for that experiment (W) over the comb growth for a control experiment (W_c) run simultaneously, in which the chicks were untreated (columns 5 and 6). This increase (W—W_c), the comb weight corrected for controls, is given in column 7. It is apparent from the data that the response obtained in any one experiment with the smaller volume of vehicle is in each case greater than the response in the corresponding experi-

ment with the larger volume. Each of these response values has likewise been calculated as per cent of the corresponding control value and is given in column 8. The improvement in response resulting from reduction in volume of sesame oil (IR, column 9) is therefore measured by the difference of these paired percentage values. These "improvement" values are uniformly positive, corresponding to the greater response with 0.02 cc of oil than with 0.05 cc. They vary in magnitude from 43% to 108% of the corresponding control comb weight. The mean improvement is 67.9%, with a standard deviation of 5.4, and is based on a total of 710 chicks: 277 treated with the larger volume of oil, 278 with the smaller volume, and 155 untreated controls. A second group of experiments, identical with the foregoing but restricted to the dosage range 1-9 y inclusive, has also been performed. This series employed a total of 308 chicks, of which 238 were treated and 70 were controls. The results were similar to those in the foregoing series.

It may be concluded, therefore, that this further reduction in volume of vehicle effects a further increase in the comb growth response of baby chicks. The only explanation which we can offer at the present time for this improvement is that the smaller the volume, the greater is the proportion of androgen solution utilized on the comb surface instead of being diverted to adjacent, less-sensitive head areas.

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

11553

Improvement in Chick Comb Response to Androsterone Obtained with Alcohol as Vehicle.*

EMANUEL KLEMPNER, ROBERT T. FRANK AND FRANKLIN HOL-LANDER.

From the Laboratories of the Mount Sinai Hospital, New York

In our studies of the bioassay method for androgens, utilizing the comb of the baby chick, it has appeared that further improve-

^{*} This investigation was supported in part by a grant from the Friedsam Foundation.

Effect of Further Reduction in Vo of Androgens L

Series d No. (1)	drogen osage (7) (2)	Vol. of oil (ec) (3)	No. of chicks (4)	Treatichick W (m; (5)	
27	10	.05 .02	25 25	34.0 43.6	
28	10	.05 .02	30 30	31.1 42.2	
29 30	10 10	.05 .02 .05	30 30 29	31.0 48.3 35.6	
26	12	.02 .05	29 15	48.8 43.1	
26	15	.03 .03	15 14	55.9 45.7	
28	15	.02	15 30	60.2 39.2	
29	15	.02 .05	30 30	50.8 38.1	
25	30	.02 .03	30 25	53.3 59.2	
27	30	.02 .05	25 24	67.6 49.1	
	50	.02 .05	24 25	69.0 71.0	
20	UV	.02	25	77.9	

*Figures in parentheses No. of chicks. $W-W_c$ 1 Response 29 % of $W_c = \frac{W-W_c}{W_c}$ 2 Improvement (IR) as % of $W_c = -\frac{W_c}{W_c}$

I, using mean comb weight for each gr sex, which had been treated in the same from 10 to 50 γ. The response to treatme measured by the increase in comb grown over the comb growth for a control extaneously, in which the chicks were un This increase (W—We), the comb weighted in column 7. It is apparent from obtained in any one experiment with the is in each case greater than the response

TABLE I.

Comparison of Alcohol and Sesame Oil as Vehicle in Bioassay of Androgens by
the Chick Comb Method.

				Mean co	omb wt	Respo andr applic (comb wt	Improve-	
A	ndroge	n	No.	Treated	Control		ntrols)	in
	dosage		of	chicks	chicks			response
No.	(y)		chicks	W (mg)	W_c (mg)	$(W-W_c)$	% of W	
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
\$39	10	Oil	18	48.1	23.6	24.5	104	+ 93
		Alcohol	17	70.2	(21)*	46.6	197	
S40	10	Qil	18	38.4	19.2	19.2	100	+155
		Alcohol	18	68.3	(37)	49.1	255	
S39	20	Oil	17	61.1	23.6	37.5	158	+152
		Alcohol		96.9	(21)	73.3	310	
838	30	Oil	18	48.1	21.7	26.4	122	+182
		Alcohol		87.8	(25)	66.1	304	
S39	30	Oil	16	68.5	23.6	44 .9	190	+134
		Alcohol		100.4	(21)	76.8	324	
840	30	Oil	18	61.3	19.2	42.1	219	+172
		Alcohol		94.6	(37)	75.4	391	
S39	40	Oil	17	71.2	23.6	47.6	201	+167
		Alcoho		110.8	(21)	87.2	368	
839	50	Oil	18	77.9	23.6	54.3	229	+206
		Alcoho		126.8	(21)	103.2	435	
\$40	50	Oil	18	84.6	19.2	65.4	339	+140
		Alcoho	1 18	111.3	(37)	92.1	479	
								a = 155.7%
							σ_{M}	$=\pm10.1\%$

*Figures in parentheses are No. of chicks.

1 Response as % of
$$W_c = \frac{W - W_c}{W_c} \times 100$$

² Improvement (IR) as % of W_c =
$$\frac{W_{alc} - W_{oll}}{W_{c}} \times 100$$

chicks (W, column 5) and that of the untreated control group in the same experiment (W, column 6). For comparative purposes, these "response" values are also given as per cent of the corresponding control weight (column 8). Then, the improvement in response obtained by the use of alcohol as compared with oil in any one pair of experiments is given by the difference between these percentage response values (IR, column 9). These "improvement" values are consistently in favor of the alcohol as vehicle, and vary from 93 to 206% of the corresponding control comb weight. The mean of these 9 IR values is 155.7% with a standard deviation of 10.1. This mean is based on a total of 538 chicks: 158 treated with oil, 160 treated with alcohol and 220 untreated controls.

It was mentioned above that the alcohol solutions were employed in daily volumes of 0.02 cc as compared with 0.05 cc for the sesame

ment in the method might be attained by utilizing some vehicle for the androgens other than sesame oil. We have been aware of the fact that an oily vehicle entails certain disadvantages: (1) It has a tendency to spread over the head feathers of the chick and thus cause a loss of variable amounts of the hormone by diversion from the comb. (2) As a solvent, oil is unsatisfactory particularly for the gummy residues frequently obtained in extracting androgens from urine. (3) The oil itself is not absorbed, as has been shown histologically by Soloway, Hansen and McCahey,1 but the hormone is absorbed selectively by diffusion out of the vehicle instead of along with it. (4) The high viscosity of an oily vehicle is a distinct source of error in the preparation of quantitative solutions of the androgens. Other liquids which have been employed in place of sesame oil are tricaproin (Hall and Dryden2), 60% alcohol for estrogens (Ito, Hajazu and Kon3), and 96% alcohol for testosterone (Zondek and Sulman⁴). The latter also mention the possibility of using benzol, ether, benzene and acetone but report no comparative studies with these solvents. Since 95% alcohol tends to overcome the undesirable features of sesame oil enumerated above, we have compared the two vehicles with respect to the comb growth response elicited by identical doses of androsterone. The procedure was similar to that described in the preceding paper (Klempner, Hollander and Frank⁵), wherein we compared the response elicited by the same dose of androsterone in paired experiments in which the only variable was the volume of sesame oil employed. In the present investigation, however, the paired experiments differed from each other in that the first employed 0.05 cc of sesame oil and the second 0.02 cc of 95% alcohol. A comparison of the relative efficiencies of the two liquids for the purpose of these investigations would have been simplified by the use of identical volumes, but—as will be shown below—this difference in volume can be canceled out of the final result without undue complications.

The data for 9 such pairs of experiments are presented in Table I. The response in any one experiment (column 7) is measured by the difference between the mean comb weight of the group of treated

¹ Soloway, D., Hansen, L. P., and McCahey, J. F., Proc. Soc. Exp. Biol. AND MED., 1939, 41, 547.

² Hall, S. R., and Dryden, L. P., Proc. Soc. Exp. Biol. and Med., 1939, 41, 378.

³ Ito, M., Hajazu, S., and Kon, T., Znbl. Gyn., 1937, 61, 1094.

⁴ Zondek, B., and Sulman, F., PROC. Soc. Exp. Biol. And Med., 1939, 40, 633.

⁵ Klempner, E., Hollander, F., and Frank, R. T., Proc. Soc. Exp. Biol. and Med., 1940, 44, 631.

TABLE I.

Comparison of Alcohol and Sesame Oil as Vehicle in Bioassay of Androgens by
the Chick Comb Method.

				Mean co	omb wt	andı appli	onse to rogen cation corrected	Improve-
Androgen		No.	Treated Control			ntrols)	in	
	dosage		of	chicks	chicks			response
No.	(_Y)	Vehicle	chicks	W (mg)	W_{c} (mg)	$(\mathbf{W} \cdot \mathbf{W}_{\mathbf{c}})$	% of W	1 (IR) 2
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
S39	10	Oil	18	48.1	23.6	24.5	104	+ 93
		Alcohol	. 17	70.2	(21)*	46.6	197	
S40	10	Oil	18	38.4	19.2	19.2	100	十155
		Alcohol	18	68.3	(37)	49.1	255	
S39	20	Oil	17	61.1	23.6	37.5	158	+152
		Alcohol	17	96.9	(21)	73.3	310	
838	30	Oil	18	48.1	21.7	26.4	122	+182
		Alcohol	18	87.8	(25)	66.1	304	
S39	30	Oil	16	68.5	23.6	44.9	190	十134
		Alcohol	l 18	100.4	(21)	76.8	324	
840	30	Oil	18	61.3	19.2	42.1	219	+172
		Alcoho	l 18	94.6	(37)	75.4	391	
S39	40	Oil	17	71.2	23.6	47.6	201	+167
		Alcoho		110.8	(21)	87.2	368	
S39	50	Oil	18	77.9	23.6	54.3	229	+206
		Alcoho		126.8	(21)	103.2	435	
\$40	50	Oil	18	84.6	19.2	65.4	339	+140
		Alcoho	l 18	111.3	(37)	92.1	479	
								2 = 155.7%
							σ_{M}	$=\pm 10.1\%$

*Figures in parentheses are No. of chicks.
$${}^{1}\text{Response as }\% \text{ of } \mathbb{W}_{\mathbf{c}} = \frac{\mathbb{W} - \mathbb{W}_{\mathbf{c}}}{\mathbb{W}_{\mathbf{c}}} \times 100$$

2
 Improvement (IR) as % of W $_{\rm c} = \frac{{\rm W}_{\rm alc} - {\rm W}_{\rm oil}}{{\rm W}_{\rm c}} \times 100$

chicks (W, column 5) and that of the untreated control group in the same experiment (W, column 6). For comparative purposes, these "response" values are also given as per cent of the corresponding control weight (column 8). Then, the improvement in response obtained by the use of alcohol as compared with oil in any one pair of experiments is given by the difference between these percentage response values (IR, column 9). These "improvement" values are consistently in favor of the alcohol as vehicle, and vary from 93 to 206% of the corresponding control comb weight. The mean of these 9 IR values is 155.7% with a standard deviation of 10.1. This mean is based on a total of 538 chicks: 158 treated with oil, 160 treated with alcohol and 220 untreated controls.

It was mentioned above that the alcohol solutions were employed in daily volumes of 0.02 cc as compared with 0.05 cc for the sesame

oil. We have shown elsewhere (Klempner, Hollander and Frank⁵) that even with the same solvent such a difference in volume may produce a considerable increase in comb response; for 11 experiments with a range in dosage of 10-50 y, the mean IR-value was $67.9 \pm 5.4\%$. However, in the present investigation the mean IR value is $155.7 \pm 10.1\%$. The difference between these two is 87.8% with a standard deviation of 11.5%. This difference is 7.6 times its standard deviation and is therefore statistically significant.

It may be concluded, therefore, that the improvement in comb growth response obtained in these experiments resulted from the substitution of alcohol for sesame oil, apart from the diminution in volume of vehicle. Such improvement may be ascribed to the rapid evaporation and absorption of the alcohol, with consequent diminution in loss by spreading to less responsive areas. It is also possible that the use of alcohol increases the rate of absorption of the androgen itself by the comb surface, but as yet we have no direct evidence of this.

We desire to express our thanks to Dr. Erwin Schwenk of the Schering Corporation of New Jersey for supplying us with the androsterone used in this investigation.

11554

Prevention of Nutritional Muscular Dystrophy in Suckling E-low Rats with Alpha-tocopherol and Related Substances.*

HERBERT M. EVANS AND GLADYS A. EMERSON.

From the Institute of Experimental Biology, University of California, Berkeley,

California.

That alpha-tocopherol was effective in preventing the dystrophy that appears in the suckling young of vitamin E-low mothers was first shown by Barrie, Demole and Pfaltz, and Goettsch and Ritzmann. Goettsch and Ritzmann found that FeCl3-treated wheat

^{*} Aided by grants from the Research Board and the Department of Agriculture of the University of California, the Rockefeller Foundation and Merck and Company, Rahway, New Jersey. The following materials were generously contributed: alpha-tocopherol by Merck and Company, Rahway, New Jersey. Assistance was rendered by the Works Progress Administration, Official Project No. 65-1-08-62, Unit A-5.

¹ Barrie, M. M. O., Nature, 1938, 142, 799.

² Goettsch, M., and Ritzmann, J., J. Nutr., 1939, 17, 371.

³ Demole, V., and Pfaltz, H., Schweiz. Med. Wochenschrift, 1939, 69, 123.

germ oil (which had no activity as vitamin E in the cure of sterility) likewise had anti-dystrophic activity.

We are able to confirm the findings of Barrie, Demole and Pfaltz, Goettsch and Ritzmann as to the anti-dystrophic activity of alpha-

tocopherol.

Young female rats reared on our vitamin E-low diet 427⁴ and of proved sterility were bred for their second gestation and were given a single dose of 3 mg of natural alpha-tocopherol on the day of finding sperm. The litters resulting from these pregnancies were reduced to 6 young. In the first group of test animals the mothers were given 6 mg of alpha-tocopherol by stomach tube on the day of littering. They were allowed to suckle 3 of their own young and 3 foster young of the control mothers which received the solvent for the alpha-tocopherol (ethyl laurate). Likewise, the control rats were allowed to suckle 3 of their own young and 3 from the experimental animals. Six mg of alpha-tocopherol was almost adequate to prevent the dystrophy that would otherwise have developed toward the end of the lactation period. Two animals, however, in this group exhibited a slight stiffness (Table I). Ten mg of alpha-tocopherol appeared to be adequate to prevent the dystrophy.

TABLE I. Prevention of Muscular Dystrophy in Suckling E-low Rats with α -tocopherol.

Treatment	No. of young	Dystrophic or dead Days 15-25
Mother received 6 mg a-tocopherol (in ethyl laurate) on day of littering	30	2 (slight stiffness)
Mother received ethyl laurate only	64	64
Mother received 10 mg a-tocopherol on day of littering	41	0
Young received 1 mg a-tocopherol daily from day 10	13	0
Young received ethyl laurate only	12	6
Young received 3 mg a-tocopherol daily from day 15	12	0
Young received ethyl laurate only	9	6
Young received 3 mg a-tocopherol daily from day 18	30	25
Young received ethyl laurate only	25	19

In a second experiment the young were given alpha-tocopherol: one group received 1 mg daily from day 10; a second group received 3 mg daily from day 15; and a third group, 3 mg daily from day 18. The results of this experiment demonstrate that alpha-tocopherol when administered as late as day 15 of lactation was effective in preventing the paralysis but that the young receiving the alphatocopherol from day 18 were not protected. It can be seen that not all the ethyl laurate treated young developed dystrophy although this was the case when the mothers were so treated. An explanation

⁴ Emerson, G. A., and Evans, H. M., J. Nutr., 1937, 14, 169.

may be afforded by the necessary conditions of the experiment for young in the same litter were fed the alpha-tocopherol by dropper and slight oral residues could have been licked off by litter mates.

Following the observation of Goettsch and Ritzmann demonstrating the activity of FeCl₃-treated wheat germ oil in preventing the dystrophy, we fed substances related chemically to alphatocopherol (I).

II-2,2,5,7,8 pentamethyl 6 hydroxy chromane has the same ring structure as alpha-tocopherol. The side chain of alpha-tocopherol is derived from phytol (III),

$$CH_3$$
 CH_3 CH_3 CH_3 CH_4 CH_2 CH_3 CH_4 CH_5 CH_5 CH_5 CH_5 CH_5 CH_5 CH_5

III-Phytol

Fernholz obtained the gamma lactone (IV)

IV-gamma lactone (Fernholz)

on oxidation of alpha-tocopherol. All of these substances were inactive whether fed to the young from day 15 or to the mothers on the day of littering (Table II).

The resemblance in structure between vitamin K1 (V)

and alpha-tocopherol suggested that this substance might act as vitamin E but vitamin K_1 was also found to be inactive in the prevention of muscular dystrophy.

TABLE II. Failure to Prevent Muscular Dystrophy in Suckling E-low Rats with Substances Related to α -tocopherol

Treatment	No. of young	Dystrophic or dead Days 15-25
Young received 3 mg phytol daily from day 15	16	14
Young received 3 mg 2,2,5,7,8, penta methyl 6 hydroxy chromane daily from day 15 Young received 3 mg gamma lactone (Fernholz)	20	13
from day 15	16	13
Young received ethyl laurate only	52	45
Mother received 15 mg 2,2,5,7,8 penta methyl 6 hydroxy chromane on day of littering	54	53
Mother received 15 mg vitamin K1 on day of littering	18	17
Mother received ethyl laurate only	28	26
Mother received 15 mg gamma lactone (Fernholz) from day 15	24	24

Summary. The dystrophy that almost invariably appears toward the end of the lactation period in the suckling young of vitamin E-low mothers can be prevented by the administration of 10 mg of alphatocopherol to the mother on the day of littering or the feeding of 1 mg daily to the young from day 10 or 3 mg from day 15. The administration of 3 mg of alphatocopherol daily from day 18 was ineffective. The following compounds related chemically to alphatocopherol were tested for anti-dystrophic activity and found inactive: 2,2,5,7,8-penta-methyl, 6 hydroxy chromane, phytol, gamma lactone and vitamin K₁.

11555 P

Reimplantation and Transplantation of Eyes in Anuran Larvae and Fundulus heteroclitus.*

L. S. STONE.

From the Department of Anatomy, Yale University School of Medicine.

These experiments were undertaken on anurans and fishes to compare the results with those obtained from a series of studies on the grafted eyes of urodeles.¹⁻³ In one group the functional eye in

^{*} Aided by grants from the John and Mary R. Markle Foundation and the Fluid Research Fund of Yale University School of Medicine.

¹ Stone, L. S., J. Exp. Zool., 1930, 55, 193.

² Stone, L. S., and Cole, C. H., PROC. Soc. EXP. BIOL. AND MED., 1931, 29, 176. 3 Stone, L. S., Zaur, I. S., and Farthing, T. E., PROC. Soc. EXP. BIOL. AND MED.,

^{1934, 31, 1082.4} Stone, L. S., Ussher, N. T., and Beers, D. N., J. Exp. Zool., 1937, 77, 13.

⁵ Stone, L. S., and Chace, R. R., PROC. Soc. Exp. BIOL. AND MED., 1937, 36, 830.

may be afforded by the necessary conditions of the experiment for young in the same litter were fed the alpha-tocopherol by dropper and slight oral residues could have been licked off by litter mates.

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$$CH_3$$
 CH_3 CH_3 CH_3 CH_3 CH_4 CH_2 CH_3 CH_4 CH_5 CH_5

III-Phytol

Fernholz obtained the gamma lactone (IV)

IX-gamma lactone (Fernholz)

on oxidation of alpha-tocopherol. All of these substances were inactive whether fed to the young from day 15 or to the mothers on the day of littering (Table II).

The resemblance in structure between vitamin K1 (V)

$$CH_3$$
 CH_3 CH_3 CH_3 CH_3 CH_2 CH_2 CH_3 CH_3 CH_4 CH_2 CH_3 CH_4 CH_4 CH_5 CH_5 CH_6 CH_6

and alpha-tocopherol suggested that this substance might act as vitamin E but vitamin K_1 was also found to be inactive in the prevention of muscular dystrophy.

Ten eyes were reimplanted in the orbit in Rana clamitans larvæ about 65 mm in length. Three died immediately after operation. Two eyes were severely injured at operation and were slowly resorbed by one month before the hosts metamorphosed. Five cases were sacrificed from 150 to 227 days after operation. Two of these were carried to metamorphosis and 3 were killed from 53 to 80 days after metamorphosis. All eyes and their pupils were slightly smaller than normal at the end of the experiment.

Histological studies showed that no degeneration was taking place in the eye when the hosts were killed. All retinæ were slightly thinner than normal. They showed a reduction in the number of ganglion cells and the inner nuclear zone was not as deep as the normal. In every case the small optic nerve stump at the bulb did not penetrate further than the choroid coat. The optic nerve was completely absent from the bulb to the chiasma. The lens was slightly smaller but normal histologically. All other structures appeared normal in both the living state and in histological preparations. The results in these older larvæ seem to be about the same as in those of the much younger R. pipiens.

11556

Occurrence of Riboflavin in Tubercle Bacillus.*

HAROLD R. STREET; AND R. E. REEVES.; (Introduced by George R. Cowgill.)

From the Department of Chemistry and the Laboratory of Physiological Chemistry, Yale University, New Haven.

It has been known for several years that aqueous extracts of the tubercle bacillus exhibit yellow fluorescence in ordinary light. We have attempted the purification and characterization of this yellow pigment. Extracts of partly defatted tubercle bacilli made with 25% alcohol showed a yellow fluorescence in ordinary light and a beautiful blue fluorescence in ultraviolet light. The formation of lumiflavin upon alkaline irradiation indicated the presence of a

^{*} Aided by grants from the Research Committee of the National Tuberculosis Association and from the Rockefeller Foundation.

f Vitamin Research Fellow at Yale University, 1936-38.

[‡] Holder of a National Tuberculosis Association Fellowship at Yale University, 1936-37.

Fundulus heteroclitus, 4 to 6 cm in length, was grafted in the orbit (32 reimplants and 20 transplants). For several days, or in some cases for weeks, the operated eye appeared perfectly normal. Circulation in most cases returned in 2 or 3 days and ocular movement was present in 10 days. The lens in some cases was slightly opaque as early as the tenth day. It usually broke down rapidly and in the living eye during the first or second month it appeared as a white gelatinous mass protruding through the pupil.

At the end of a month the pupil and the eye became slightly smaller. The iris began to show pigment changes and during the second month most eyes were slowly resorbed. Throughout the

experiment the cornea never became opaque.

The animals were sacrified 1 to 95 days after operation. Histological sections showed that the central region of the retina degenerated rapidly, beginning on the second day. The rod and cone cells in this area were slightly more resistant than other layers. The ciliary region, so resistant in urodeles (opus cited), was still a complete ring of cells on the third and fourth weeks when the rest of the retina was a mass of débris. In one unusual case (2 reimplanted eyes on the same host) both eyes on the ninety-fifth day appeared much like the normal. The lenses were clear and undegenerated and the retinæ had not undergone extensive degeneration.

Eighty-two eyes were reimplanted in the orbit in Rana pipiens larvæ 18 mm in length. Thirty eyes sloughed out in 24 hours after operation. Fifty-two healed in place and circulation returned as early as the second day. Forty-three specimens were sacrificed from one to 96 days after operation for histological studies. Several were preserved one month after metamorphosis. The growth and size of many eyes equalled the normal while some were slightly smaller. Ocular movement was observed as early as the tenth day. The cornea was never opaque and in only 2 instances (specimens killed on the third and fourth days) was the lens cloudy. Histological studies showed that in the region of the optic nerve the retina possessed varying amounts of degenerating cells during the first week. A number of cells were also lost throughout the ganglion cell layer. Some cells were lost locally in the inner nuclear zone while the rod and cone cells seemed to be normal. During the second week the optic nerve degenerated as far as the chiasma. From the second week to one month after metamorphosis there was no further sign of degenera-If injuries were not extensive at operation the retina healed early but always carried the scar. These eyes showed very little capacity to regenerate even an optic nerve. This is quite different from the results obtained in urodeles (opus cited).

to Kuhn, Wagner-Jauregg and Kaltschmitt.⁶ On this basis, it was calculated that the original dry bacilli contained 36.6 mg of flavin per kilo.

Portions of dry defatted human strain A-12 and the avian tubercle bacillus, Hygienic Laboratory No. 531, both grown on the Long³ synthetic medium, were also examined for flavin pigments. The extraction and the estimation as lumiflavin were carried out as above. The values found corresponded to 13.0 mg of flavin in the human strain A-12 and 19.3 mg in the avian bacilli per kilo of original dry bacteria. These values can only be regarded as minimal. Living bacilli would undoubtedly contain larger quantities of flavin.

Concentration of Flavin. A considerable amount of polysaccharide and other solid matter was removed from the aqueous solution of the A-14 extract by precipitation with three parts acetone and one part alcohol. This precipitate also contained much of the nonflavin fluorescing pigments which have not yet been adequately investigated.

The supernatant solution was concentrated to 800 cc, adjusted to 0.1 N with H₂SO₄ and the flavin was adsorbed by shaking with fuller's earth. After elution with pyridine-methanol-water the concentration was continued by formation of the silver salt in the usual manner. Removal of the silver left 0.487 g of solid material containing 2 mg of flavin, determined by the irradiation procedure.

Biological Assay. The crude flavin concentrate was assayed as follows: A litter of eight 21-day-old albino rats were fed ad libitum a basal diet, complete except for the vitamin B complex, of the following composition: casein 18, sucrose 73, salt mixture, Osborne and Mendel, 4, Crisco 2, and cod liver oil 3 parts. In addition, each rat received daily a rice polish extract (tikitiki) equivalent to 1 g of rice polish. We have shown that this tikitiki contains all the members of the vitamin B complex required by rats except riboflavin, of which only traces are present.

When growth had nearly ceased, one group of rats was fed the bacterial flavin, one group was given crystalline riboflavin, and a third group was continued on the diet without further supplement. The growth curves are shown in Fig. 1 and indicate that the bacterial flavin possessed the same biological activity as crystalline riboflavin.

Kuhn, Wagner-Jauregg, and Kaltschmitt, Ber., 1934, 67, 1452.
 Street and Cowgill, Am. J. Physiol., 1939, 125, 323.

[§] Obtained from the Borden Company, Bainbridge, N. Y., as crystalline lactoflavin, P X grade.

flavin-like pigment. This pigment was partially purified, as described below, and fed to rats receiving a diet lacking riboflavin. The resulting growth response indicated that the bacterial flavin was the well known water-soluble vitamin, riboflavin.¹

At the time this work was done in 1937, there were no published reports indicating the chemical nature of this yellow pigment of the tubercle bacillus. However, since that time, Boissevain, Drea and Schultz² have announced the isolation of riboflavin from the tubercle bacillus, identified by melting point and absorption spectrum. Our work on the biological activity of the pigment may be considered to confirm its identity as riboflavin.

The several strains of tubercle bacilli used in this investigation had been grown on the Long³ Synthetic Medium in the Biological Laboratories, Sharp and Dohme, at Glenolden, Pennsylvania. The material represented the dried cell residues after extraction with alcohol, ether and chloroform.

The cell residues from tubercle bacilli, Strain A-14, had been extracted in 1934 as described by Crowder, Stodola, Pangborn, and Anderson.⁴ For the present examination 522 g of the dried cell residues were digested and extracted repeatedly with 2-liter portions of warm 25% alcohol. The combined extracts were filtered through a Chamberland filter and the clear filtrate was concentrated in vacuo to a volume of 1076 cc. This extract contained 54.8 g of solids, which were mostly polysaccharides and inorganic salts, but a small amount of protein was present, together with other undetermined constituents.

The extract showed a yellowish-green fluorescence in ultraviolet light. At pH 2 the fluorescence was green, but in the range of pH 3 to 5 it was blue when irradiated with ultraviolet light. Extraction with chloroform or other solvents either at neutral, acid or alkaline reaction did not remove any fluorescent pigment.

The amount of flavin was estimated in 2 cc of the extract by conversion to lumiflavin by irradiation in alkaline solution according to the procedure of Warburg and Christian.⁵ The lumiflavin thus produced was extracted by chloroform from the acidified solution and estimated by means of the Pulfrich spectrophotometer according

¹ Kuhn, György, and Wagner-Jauregg, Ber., 1933, 66, 576, 1034.

² Boissevain, Drea, and Schultz, Proc. Soc. Exp. Biol. and Med., 1938, 39, 481.

³ Long, Am. Rev. Tuberc., 1926, 13, 393.

⁴ Crowder, Stodola, Pangborn, and Anderson, J. Am. Chem. Soc., 1936, 58, 636.

⁵ Warburg and Christian, Biochem. Z., 1933, 266, 377.

11557

Effect of Certain Purines, and CO₂ on Growth of Strain of Group A Hemolytic Streptococcus.*

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From the Department of Bacteriology, School of Medicine, University of Pennsylvania.

Rapid luxuriant growth of the C203S strain of Group A hemolytic streptococcus, equal to that occurring in broth under comparable conditions, has been obtained on a medium of essentially known composition. The complete medium is made up as follows:

I. 40 cc of stock solution of acid hydrolyzed gelatin equivalent to 25% gelatin¹ are diluted with distilled water to 500 cc and 500 mg cystine dissolved in a few cc of dilute HCl, 3 g KH₂PO₄, 1 g Na₂HPO₄ (anhydrous), and enough 5N NaOH to bring the pH to 7.4-7.6 are added. The solution is boiled gently for 5 minutes and filtered.

II. To the filtrate are added 50 mg tryptophane, 100 mg tyrosine, 15 mg adenine sulfate, 10 mg uracil, 0.2 mg nicotinic acid, 2 mg synthetic vitamin B₀,† 0.1 mg of biotin concentrate‡ and 2 cc of salt mixture (25 g MgSO₄ · 7 H₂O, 20 mg MnCl₂ · 4 H₂O, 5 mg CuSO₄, 2 mg FeSO₄ · 7 H₂O, and 2 mg ZnSO₄ · 7 H₂O made up to 100 cc with water containing a few drops of concentrated HCl). The volume is made up to 900 cc, the solution readjusted to pH 7.4-7.6, tubed in 9 cc amounts and autoclaved at 10 lb for 10 minutes.

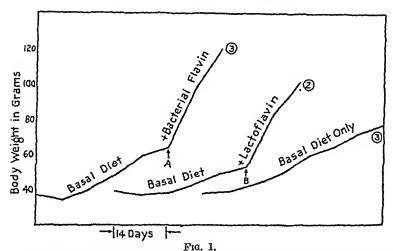
III. To each tube is added 0.1 cc of the following solutions which have been sterilized separately: 0.1 mg thiamin (vitamin B₁) per cc, 0.05 mg riboflavin per cc, 0.1 mg synthetic d-calcium pantothenate per cc, 1% neutralized thioglycollic acid containing 0.2 mg glutathione per cc and 5 mg glutamine per cc. The last three solutions

^{*} The expenses of this work have been defrayed by a generous grant from the Commonwealth Fund.

¹ Pappenheimer, A. M., Jr., and Johnson, S. J., Brit. J. Exp. Path., 1937, 18, 239.

t We are greatly indebted to Merek & Co. for generous samples of synthetic vitamin \mathbf{B}_0 and calcium pantothenate.

t We are indebted to Dr. D. W. Woolley of the Rockefeller Institute, New York, for this concentrate which was prepared according to the procedure of Woolley, D. W., McDaniel, L. E., and Peterson, W. H., J. Biol. Chem., 1939, 131, 381. From its activity in promoting growth of Clostridium butylicum Dr. Woolley estimates that approximately 4% of the material is actual biotin. (See also Peterson, W. H., McDaniel, L. E., and McCoy, E., J. Biol. Chem., 1940, 183, lxxv.)



The effect of bacterial flavin and of crystalline riboflavin on the growth of rats fed a basal diet supplemented with rice polish extract. At point A the daily administration of the bacterial flavin preparation in amount calculated to supply 40 γ of flavin was begun. At point B the daily administration of 40 γ of pure crystalline riboflavin was begun. The curves are averages. The figure in the circle by each curve represents the number of animals in the group.

In view of the uniformity in growth response of the several animals, it appears justifiable to present the results in the form of curves based on averages. While the number of animals used was necessarily small, many other tests performed in this laboratory using the same basal diet, including rice polish extract alone or supplemented with crystalline riboflavin, have yielded results practically identical with the curves shown above.

The average daily gain in weight of 3.5 g daily observed in these experiments with a daily supplement of 40 γ of riboflavin, is essentially normal growth for this strain of rats and indicates that the basal ration supplies adequate amounts of all nutritional essentials required by the rat other than riboflavin. Under these circumstances, any increased gain over that of the negative controls produced by a vitamin bearing substance should be specific for riboflavin. Since this work was completed one of us (H.R.S.) has used a ration, containing tikitiki as a source of the B complex, for riboflavin assays involving the use of over 1000 rats, with entirely satisfactory results.

Summary. The tubercle bacillus cultivated on the Long synthetic medium is capable of synthesizing fluorescent flavin pigment. This pigment, when fed to young rats, promotes growth to the same extent as a corresponding quantity of crystalline riboflavin.

TABLE I. Effect of Omitting Certain Factors from Complete Medium on Growth of C203S Strain.

Substance	Amt necessary for optimal growth	Mg bacterial N after 40 hr incubation from 10 cc × 50
Complete medium	_	10-13
No glucose	25 mg	0.1
" glutamine	500 μg	variable*
"tyrosine	1000	3.9
'' tryptophane	500 ''	0.8
", uracil	100 ''	9.9
"adenylic acid (0.4 mm CO2 tension) 100 "	0.7
" CO2 (CO2-free air)	8 mm	2.2
"thioglycollic acid	1 mg	}
" glutathione		₹0.1
" thiamin	$^4_{0.01}$ $^{\mu g}_{jj}$	3.1
" nicotinie acid	1.0 ''	1.9
'' pantothenic acid	10 ,,	0,2
'' riboflavin	0.04 ,,	0.8
" vitamin Ba	20 "	5.9-7.1†
"" biotin" concentrate	1.0 ,,	1.8
Broth (25 mg glucose in 10 cc)		10-17

^{*}No significant growth at 20 hours without glutamine. Growth at 40 hours at 8 mm CO₂ tension is variable.

†No significant growth at atmospheric CO₂ tension without vitamin B₆.

C203S for adenine or related compounds. If purine is omitted from the complete medium no growth occurs within 40 hours. Addition of adenine permits growth to occur. Adenine may be replaced by adenosine or adenylic acid, by guanine, guanosine or guanylic acid and by xanthine or hypoxanthine. It cannot be replaced by uric acid, caffein or theophylline or by the pyrimidines uracil and cytosine. Subbarow and Rane³ have reported that certain of the above purines "may be of significance" in the growth of the N.Y. No. 5 strain of hemolytic streptococcus. McIlwain has included a number of purines in his medium on general grounds and Möller has shown that adenine or guanine but not xanthine or hypoxanthine increase the growth of Lactobacillus plantarum.

The purine requirement of strain C203S was discovered before all of the other growth factors had been identified. Upon reexamining the purine requirements using the more defined medium given in detail above, it was noted that the presence of 5% carbon dioxide in the atmosphere above the culture greatly accelerated growth and the surprising observation was made that even when purine was absent growth occurred. We therefore examined the effect of carbon dioxide in some detail and in Table II are shown some of the

⁷ Möller, E. F., Z. Physiol. Chem., 1939, 260, 246.

are sterilized by filtration. Finally, 0.5 cc of 5% glucose containing 0.04% CaCl₂·2 H₂O is added to each tube.

IV. The inoculum consists of one drop of 6-8-hour broth culture of C203S which has been twice washed with saline and then made up to slight turbidity with saline. Tubes are incubated under 8 mm CO₂ tension in air for 40 hours. The amount of growth is determined with a photoelectric colorimeter and the readings correlated with standard suspensions of known bacterial nitrogen content.

The effect of omitting each of the above factors in turn from the complete medium is listed in Table I. Figures in the second column indicate the minimum amount of substance necessary for optimum growth in 10 cc of medium.

All the substances listed in Table I are essential for rapid growth with the exception of uracil. Uracil, while not essential, appears to increase growth slightly.

In the complete medium the limiting factor appears to be glucose. Addition of more glucose will increase growth proportionately until sufficient acid has been produced to kill the organisms.

The need for pantothenic acid and riboflavin agrees with the results of Rane and Subbarow, 2-3, McIlwain, 4, and Woolley and Hutchings 5 using other strains. The necessity of glutamine for rapid growth confirms the work of McIlwain et al. 6 In agreement with McIlwain we have found that glutathione is not essential provided the thioglycollic acid concentration is increased to 10-3 molar in the final medium. Our findings with respect to vitamin B₈ would also seem to confirm those of McIlwain and of Woolley and Hutchings 5 on Group D strains. Thiamin has not previously been reported essential for growth of hemolytic streptococcus. While it does not seem improbable that biotin is necessary for growth of the C203S strain, this cannot be regarded as certain until pure biotin becomes available for test. It is also possible that some other essential factor may be present as impurity in the gelatin or in the glutamine, both of which were prepared from natural sources. This seems unlikely in view of the good growth obtained.

We have been particularly interested in the requirement of strain

² Rane, L., and Subbarow, Y., PROC. Soc. EXP. BIOL. AND MED., 1938, 38, 837. 3 Subbarow, Y., and Rane, L., J. Am. Chem. Soc., 1939, 61, 1616.

⁴ McIlwaie, H., Brit. J. Exp. Path., 1939, 20, 330; Brit. J. Exp. Path., 1940, 21, 25.

⁵ Woolley, D. W., and Hutchings, B. L., J. Bact., 1939, 38, 285.

⁶ McIlwain, H., Fildes, P., Gladstone, G. P., and Knight, B. C. J. G., Biochem. J., 1939, 33, 223.

growth occurs under anaerobic conditions unless uracil is added. In the presence of oxygen uracil is non-essential.

The C203S strain of streptococcus is a powerful hemolytic strain. In our experience hemolytic titers on this medium are equivalent to those obtained in broth, provided care is taken to avoid accumulation of acid during growth. The growth and hemolysin titer may be increased by addition of more glucose to the medium and periodic neutralization of the acid formed. However, preliminary work in this direction indicates that some factor, as yet unidentified, becomes the limiting one under these conditions.

Summary. Rapid, heavy growth and hemolysin production of the C203S strain of Group A hemolytic streptococcus have been obtained on a medium of essentially known composition. In addition to factors reported by previous workers, we have found that thiamin, nicotinic acid, adenine or related purines, and an unknown factor which may possibly be biotin are necessary for growth of this strain. The relation of carbon dioxide tension to the purine requirement has been studied.

11558

Modifying Influence of Light on Chick's Comb Response to Androsterone.

WILLIAM F. STARKEY, ROBERT C. GRAUER AND ELEANOR SAIER. (Introduced by S. R. Haythorn.)

From the Department of Research in Endocrinology and Metabolism, William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh.

The assay of androgenic material by biologic methods offers definite advantages over colorimetric determinations when we consider that in measuring the 17 ketosteroids by chromogenic effect we are determining both biologically active and inactive material. In many cases the important consideration is, to what degree is biological activity present in a given specimen. In consequence of this, we made comparisons of colorimetric² and biologic determinations in some of our studies. The comb response of one-day-old

¹ Callow, N. H., Callow, R. K., Emmens, C. W., and Stroud, S. W., J. Endo., 1939, 1, 76.

² Neustadt, Rudolph, Endo., 1938, 23, 711.

TABLE II. Effect of Adenylic Acid on Growth of Strain C203S at Different Carbon Dioxide Tensions.

Carbon dioxide tension (mm Hg)	No adenylic acid		Adenylie acid, 10 μg/e	
	20 hr	40 hr	20 hr	40 hr
COofree air		0.3		2.2
0.4	0.0	0.7	1.9	6.8-8.2*
1.4	0.1	0.7	3.1	8.4
2.4	0.15	3.8	3.0	9.1
4.3	0.15	9,0	10.9	10.1
8	1.4	9.1	11.3	10.4
20	1.0	9,2	9.6	10.8
40	10.1	12.2	12.2	12.4
am CO., 730 mm nitrogen,				
no oxygen		12.5		12.5

*Growth is quite variable at atmospheric CO2 tension which has been assumed to be 0.4 mm Hg.

Note that readings at 20 and 40 hours were from different experiments in different tubes. Growth is given as milligrams bacterial nitrogen per 10 cc × 50.

Figures represent averages of at least 2 tubes.

The oxygen tension was kept constant at 120 mm throughout except in the anærobic experiments and those done at atmospheric CO₂ tensions. The gas pressure was made up to 740 mm with nitrogen in each case.

results at different CO2 tensions with and without adenylic acid. It will be noted that maximal growth when adenylic acid is present occurs within 20 hours provided the CO2 tension is 4 mm or greater, that no significant growth occurs even after 40 hours' incubation in the absence of adenylic acid when the CO2 tension is below 2 mm and that even when the CO2 tension is high, a small but consistent increase in growth is apparent at 40 hours when adenylic acid is added to the medium. It has been observed that the bicarbonate ion cannot replace carbon dioxide when no purine is present. However, in the presence of both adenylic acid and bicarbonate, any slight growth of the organisms may liberate sufficient carbon dioxide through action of the acid produced to accelerate growth. These observations on the accelerating effect of carbon dioxide are in harmony with those of other workers8 and indeed McIlwain4 grew his cultures in 5% carbon dioxide.

At present we have no clue as to the significance of these findings. Whether carbon dioxide is necessary for purine synthesis or whether purine plays a rôle in the production of carbon dioxide by the organisms cannot be decided at this time. We may point out, however, that a somewhat analogous situation has been reported in the case of Staphylococcus aureus by Richardson.9 With this organism no

⁸ Gladstone, G. P., Fildes, P., and Richardson, G. H., Brit. J. Exp. Path., 1935, 16, 335.

⁹ Richardson, G. H., Biochem. J., 1936, 30, 2184.

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chicks, previously reported by Dorfman and Greulich,³ Emmens,⁴ and Frank and Klempner,⁵ was employed as the method of studying biologic response. During the preliminary plotting of our curves, to establish normal average response to known dosage, it was recognized by us that light played an extremely important rôle as a modifying factor in the weight response of the chick's comb. Though this factor has been observed in the capon,⁶ we have found no mention in the literature regarding the baby chick. It is, therefor, of paramount importance that the influence of light be recognized and taken into account by workers who have adopted the chick comb weight method for the assaying of androgens.

The 309 white leghorn male chicks used in our experiment were procured from the same hatchery on the day of hatching. All chicks used were hatched during the first 3 weeks of April. When the chicks were one day old, daily inunction of the combs was begun and continued for 6 days. The combs were removed and weighed on the day following cessation of treatment and the weights of the birds also were noted. Removal of the comb was accomplished by making lateral and posterior incisions at the base of the comb of the anesthetized chicks and by grasping the anterior end with forceps and stripping the comb off dorsally. All combs were removed by the same individual.

Daily treatment consisted of spreading 0.01 cc of sesame oil over the surface of the comb. This was easily accomplished with accuracy by the use of a micro-titrating pipette calibrated in 0.002 cc divisions and which had the tip bent at a right angle. This amount of oil contained from 0.5 to 50.0 γ of androsterone* and was applied at each inunction to groups of from 5 to 14 chicks. The doses used in the various groups are indicated in Fig. 2 and 3. The oil applied was absorbed within about 10 minutes, during which period the chicks on each dose were kept separated to prevent contamination through contact. Control animals which received only sesame oil were kept in separate cages.

The chicks were divided into 4 groups, each group being exposed to varying degrees of light and darkness. One hundred and five

³ Dorfman, R. I., and Greulich, W. W., Yale J. Biol. and Med., 1937, 10, 79.

⁴ Emmens, C. W., Med. Res. Council "Reports on Biological Standards-V," 1939.

⁵ Frank, R. T., and Klempner, E., Proc. Soc. Exp. Biol. and Med., 1937,

⁶ Womack, E. B., Koch, F. C., Domm, L. V., and Juhn, M., J. Pharm. Exp. Therap., 1931, 41, 173.

^{*} The androsterone in this study was kindly supplied to us by Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc.

animals were maintained in a large incubator in total darkness, being brought into daylight only during the period of treatment which did not exceed one hour per day. Thirty-three chicks were placed in a room where they received very little natural light, referred to as "subdued". Ninety-one animals were placed in a large, well-lighted, airy room where both natural and artificial light was adequate and in which the cage was so located that all the chicks received approximately the same amount of light. The last group of 100 chicks was placed in total darkness for the first 3 days of treatment and were then placed in the same room as the chicks of Group 3 for the second 3 days. Although a mortality of approximately 50% was noted among the groups kept under deficient lighting conditions, only 5% died when the animals were kept in adequate light. The numbers of animals referred to above represent the surviving members of each group.

The effect of the various light conditions may be seen in Fig. 1 where the average body weights of each group, taken at the seventh day, have been presented. The inhibition to body growth, due presumably to inadequate light, is clearly seen where a direct correlation between the amount of light the chick received and the body weight is made. The influence of light alone on the body growth of chicks had been previously reported. The body weights of each

NORMAL LIGHT - 61.5 gm.

SUBDUED LIGHT - 53.3 gm.

DARK 3 days- LIGHT 3 days- 51.8 gm.

DARK - 45.6 gm.

AVERAGE BODY WEIGHTS OF CHICKS UNDER VARIOUS CONDITIONS OF LIGHT Fig. 1.

⁷ Bovie, W. T., Boston Med. and Surg. J., 1925, 192, 1035.

chicks, previously reported by Dorfman and Greulich,³ Emmens,⁴ and Frank and Klempner,⁵ was employed as the method of studying biologic response. During the preliminary plotting of our curves, to establish normal average response to known dosage, it was recognized by us that light played an extremely important rôle as a modifying factor in the weight response of the chick's comb. Though this factor has been observed in the capon,⁶ we have found no mention in the literature regarding the baby chick. It is, therefor, of paramount importance that the influence of light be recognized and taken into account by workers who have adopted the chick comb weight method for the assaying of androgens.

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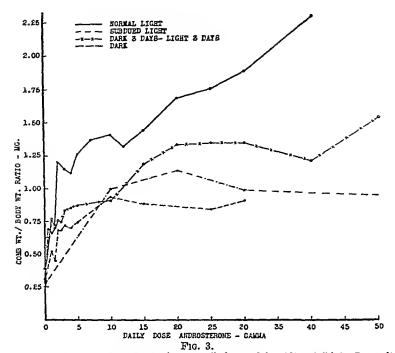
³ Dorfman, R. I., and Greulich, W. W., Yale J. Biol. and Med., 1937, 10, 79.

⁴ Emmens, C. W., Med. Res. Council "Reports on Biological Standards-V,"

⁵ Frank, R. T., and Klempner, E., PROC. Soc. Exp. Biol. and Med., 1937, 36, 763.

⁶ Womack, E. B., Koch, F. C., Domm, L. V., and Juhn, M., J. Pharm. Exp. Therap., 1931, 41, 173.

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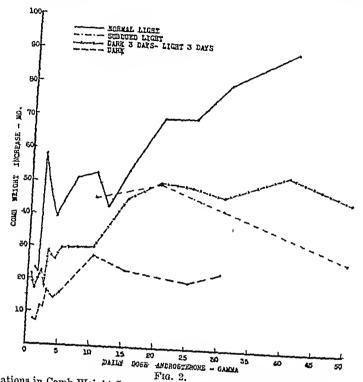
Comb Weight Body Weight Ratio Curve as Influenced by Altered Light Intensity.

In Fig. 3, the ratios of average comb weight over the average body weight have been plotted against the daily dose. Consideration of the comb weight in relation to body weight tends to remove some of the variations occurring when the comb weights alone are considered.

It becomes evident that two variable factors influence the growth of the combs of chicks; namely, the amount of androgenic material employed, and the intensity of the light to which the chicks are exposed. The light factor must, therefore, be made constant and of adequate intensity. With the changing seasons of the year, this must be borne in mind and due corrections made if a constant response is to be secured and sources of error are to be reduced to a minimum. Disregard of the amount of light available to the chicks may easily explain the discrepancies in results obtained by various workers. It is not within the province of this report to postulate whether the stimulating influence of androsterone is inhibited by the lack of light, but it is evident there is sufficient physiological inhibition to the animal organism so that it acts as a factor in preventing proper response.

Summary. Exposure to varying degrees of light influenced the

of our groups were plotted against the dosage of androsterone but failed to indicate any correlation with the treatment. This graph has been omitted.



Variations in Comb Weight Increase as Influenced by Altered Light Intensity.

In Fig. 2, the average comb weight increase of the treated animals over the control comb weights has been plotted against the daily dosage of androsterone. A comparison of the curves in this figure shows the effect of exposing the chicks to varying degrees of light intensity while employing the same dose of androsterone. There is a slight response of the animals maintained in the dark as compared with those in normal light, with the groups exposed to inadequate light occupying a median position. It is also of interest to observe that the animals kept under deficient light conditions failed to show a corresponding comb weight increase with the application of higher concentrations of androsterone as did the groups in normal light. The curves in the "deficient light groups" tend to reach a plateau. The curve of the groups in normal light is still fairly steep at a daily dosage of 40 γ , a fact rather important if this method is to be considered for assay of unknown extracts.

level of glomerular filtration and that the filtration rate is fairly constant at low and high rates of urine formation.4

Alving and Miller have shown that after the intravenous injection of inulin (10 g in adults) the plasma inulin concentration, plotted logarithmically against time, decreased at first in a curvilinear manner, later falling, however, in a linear or almost linear relationship The curvilinear relationship marks, presumably, the with time. period of equilibration between the blood and plasma and the extracellular fluids. The straight line relationship follows because once equilibrium has been established the rate of fall of the plasma inulin level is determined by the rate at which inulin is cleared from the plasma by the kidneys. Since inulin is quantitatively excreted in the urine after intravenous injection, and since, as previously stated, the inulin clearance is independent of the rate of urine formation, the rate of fall of the inulin in the plasma after the straight line relationship has been established should have a direct relationship with the clearance, and it should be possible to relate the slope of this line to the clearance. The determination of the slope of this line is the basis for the method here proposed for the estimation of glomerular filtration rate.

The method is applied as follows: a blood sample is obtained which serves to correct subsequent blood inulin analyses for the non-inulin chromogenic material. 0.15 g of inulin per kilo body weight is injected intravenously. At approximately 2 and 3 hours after the completion of the injection blood samples are drawn. The exact times of the injection and the drawing of the second and third samples are noted. The inulin content of the 2 samples of blood is determined. On semi-logarithm paper the 2 blood samples are plotted on the logarithmic coördinate against time on the linear coördinate. A straight line is drawn between these 2 points, and the slope of the line determined by the formula:

$$slope = \frac{K - \log C}{t}$$

where K is a constant whose value is determined by extending the line to zero time and taking the log of the concentration at this point; C is the plasma inulin concentration in mg % at any given time, t, in minutes, after the inulin injection.

The relation of the slopes of the lines obtained above to inulin clearance as actually determined is shown in Fig. 1. The dots rep-

⁴ Smith, H. W., The Physiology of the Kidney, Oxford University Press, New York, 1937.

weight response of male chicks' combs to which androsterone had been applied by inunction. The weight response of the combs to androgenic stimulation is considerably greater in birds receiving normal daylight than in those kept in the dark or in inadequate light. Body weights of the various groups indicate a lack of growth which is in direct correlation to the lack of light. The importance of exposing the test birds to an adequate and a constant source of light is emphasized if this method is to be used for the assay of unknown androgens.

11559

Renal Physiology in Infants and Children: I. Method for Estimation of Glomerular Filtration Rate.

HENRY L. BARNETT. (Introduced by Peter Heinbecker.)

From the Department of Pediatrics, Washington University School of Medicine,
and the St. Louis Children's Hospital.

The more exact methods for estimation of kidney function have been quite successfully employed in older children, i but they have not been very widely applicable to infants because of the obvious difficulties in obtaining complete urine specimens. Schoenthal and his coworkers² studied the urea clearance in 9 normal infants ranging in age from 2 to 111/2 months and concluded that the urea clearance corrected for surface area agreed with the values observed by Van Slyke and his coworkers for older children and adults, which indicated to them that renal function measured by the ability to excrete urea is as well developed in infants as in later life. studies d'd not include, however, the investigation of renal function during very early postnatal life. The method to be described here originated in an attempt to study the renal physiology during the newborn period. The single injection inulin clearance test proposed by Alving and Miller3 seemed especially applicable to this problem. Inulin clearances seemed preferable to urea clearances for this study since it has been well established that the inulin clearance is at the

Cullen, G. E., Nelson, W. E., and Holmes, F. E., J. Clin. Invest., 1935. 14, 563.
 Schoenthal, L., Lurie, D., and Kelly, M., Am. J. Dis. Child., 1933, 45, 41.

³ Alving, A. S., and Miller, B. F., A Practical Method for the Measurement of Glomerular Filtration Rate (Inulin Clearance), to be published.

level of glomerular filtration and that the filtration rate is fairly constant at low and high rates of urine formation.

Alving and Miller have shown that after the intravenous injection of inulin (10 g in adults) the plasma inulin concentration, plotted logarithmically against time, decreased at first in a curvilinear manner, later falling, however, in a linear or almost linear relationship with time. The curvilinear relationship marks, presumably, the period of equilibration between the blood and plasma and the extracellular fluids. The straight line relationship follows because once equilibrium has been established the rate of fall of the plasma inulin level is determined by the rate at which inulin is cleared from the plasma by the kidneys. Since inulin is quantitatively excreted in the urine after intravenous injection, and since, as previously stated, the inulin clearance is independent of the rate of urine formation, the rate of fall of the inulin in the plasma after the straight line relationship has been established should have a direct relationship with the clearance, and it should be possible to relate the slope of this line to the clearance. The determination of the slope of this line is the basis for the method here proposed for the estimation of glomerular filtration rate.

The method is applied as follows: a blood sample is obtained which serves to correct subsequent blood inulin analyses for the non-inulin chromogenic material. 0.15 g of inulin per kilo body weight is injected intravenously. At approximately 2 and 3 hours after the completion of the injection blood samples are drawn. The exact times of the injection and the drawing of the second and third samples are noted. The inulin content of the 2 samples of blood is determined. On semi-logarithm paper the 2 blood samples are plotted on the logarithmic coördinate against time on the linear coördinate. A straight line is drawn between these 2 points, and the slope of the line determined by the formula:

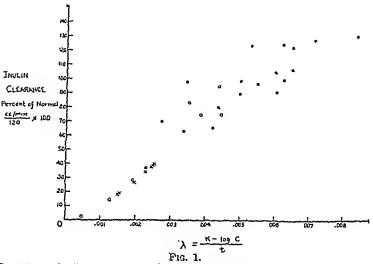
slope =
$$\frac{K - \log C}{t}$$

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The relation of the slopes of the lines obtained above to inulin clearance as actually determined is shown in Fig. 1. The dots rep-

⁴ Smith, H. W., The Physiology of the Kidney, Oxford University Press, New York, 1937.

resent data on children ranging in age from 5 to 15 years. The inulin clearances corrected for body size were done according to the technic described by Alving and Miller* and the inulin determinations by the method of Alving, Rubin, and Miller.⁵ Each point represents the average of 2 consecutive periods. The open circles represent points calculated from the data of Alving and Miller.⁴ A fairly good relationship is shown to exist between the 2 factors.

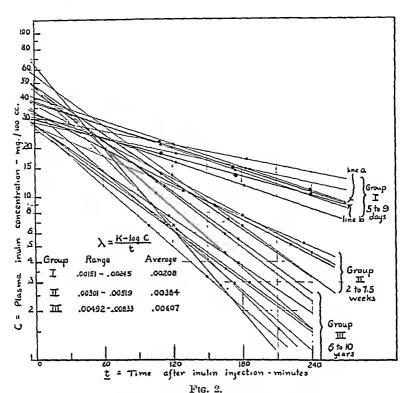


Relation of inulin clearances to slopes of lines representing fall in plasma inulin concentration following intravenous injection of inulin. Dots represent data on children from 5 to 15 years. Circles represent points calculated from the data of Alving and Miller. For explanation of crosses see text.

The slope of the line obtained as described appears to offer a fair estimation of the rate of glomerular filtration. The method would seem to be of value in instances in which the collection of urine specimens is very difficult and in which the error in collecting urines is probably greater than the errors inherent in the method. This would apply particularly to very young infants.

The application of this method to 7 apparently normal full-term newborns ranging in age from 4 to 9 days revealed slopes corresponding to inulin clearances ranging from 20 to 40% of normal (120 cc per minute). The slopes of these lines are plotted on Fig. 1 as crosses along the general line of the graph. The lines are shown as Group I in Fig. 2. That a straight line relationship in the decrease of the plasma inulin concentration, plotted logarithmically against time, does exist in newborns after an intravenous injection

⁵ Alving, A. S., Rubin, J., and Miller, B. F., J. Biol. Chem., 1939, 127, 609.

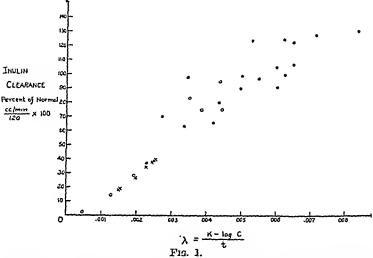


Lines obtained in different age groups by plotting plasma inulin concentrations logarithmically against time following intravenous injection of inulin.

of inulin is shown in Fig. 2 in which 3 points on the line were obtained in 2 instances (lines a and b). The lines obtained in 4 older infants ranging in age from 14 days to 71/2 weeks and apparently without kidney disease (Group II) and the lines obtained by repeated determinations on 2 children 6 and 10 years of age (Group III) are also plotted on Chart 2. The slopes of the lines obtained on Group II correspond to clearances ranging from 50 to 90% of normal, and the slopes of the lines of Group III correspond to normal These results suggest a definitely diminished inulin clearance in newborns, which rapidly disappears during early infancy, perhaps in some cases as early as the 14th day. These results correlate with the histological characteristics of the renal glomerulus in early postnatal life, a recent study of which is reported by Gruenwald and Popper,6 who showed that in embryonic life there exists a resistance against filtration due to a matting together of the glomerular loops which are invaginated in a sac of high columnar epithelium.

⁶ Gruenwald, P., and Popper, H., J. Urol., 1940, 43, 452.

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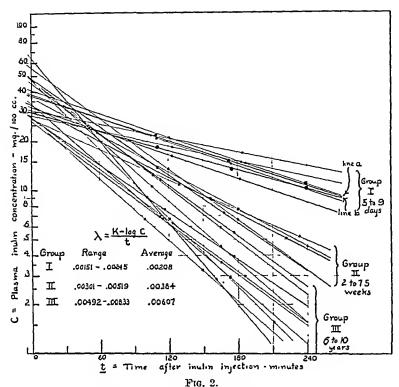


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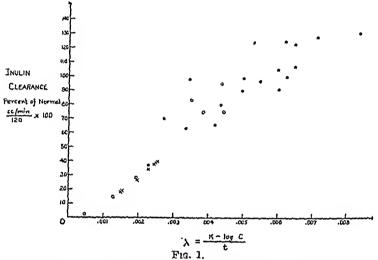


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⁵ Alving, A. S., Rubin, J., and Miller, B. F., J. Biol. Chem., 1939, 127, 609.

Experimental. The Criterion. The phenomenon of bloody tear flow in rats occurs within 2-5 minutes after an intraperitoneal injection, usually following salivation and clear tears; and it is almost instantaneous with an intravenous injection of effective doses. If salivation and clear tears are absent within 2-5 minutes after injection, usually bloody tears never flow. In the borderline cases, the naked eye may fail to recognize bloodiness in the tear, but with filter paper a slight tinge of red may be detected. In such a case, chromodacryorrhea is considered to be ±.

Animals. For intraperitoneal injections, rats of any size, sex and age are used; but for intravenous injections, young rats weighing 100-200 g are chosen. For intravenous injection, the tail is carefully washed, immersed in warm water and immediately the solution is injected into the caudal vein by means of a 27 gauge needle with a tuberculin syringe. The same rats can be used repeatedly if they are injected only once a day.

Eserine Treatment. Preliminary experiments on optimum conditions for eserinization of rats show that an intraperitoneal injection of 50 γ eserine sulfate per 100 g body weight is best for both intraperitoneal and intravenous injections of acetylcholine. In either case, an acetylcholine solution is injected after the eserine effect becomes obvious (5-10 minutes). A convenient solution for eserinization is prepared by dissolving 10 mg of eserine sulfate in 20 cc H_2O , 0.1 cc of which contains 50 γ , the exact quantity necessary for each 100 g body weight.

The Minimum Chromodacryorrhetic Doses. The chromodacryorrhetic response was determined for 4 different cases, intraperitoneal injection with and without eserine and intravenous injection with and without eserine. The minimum effective doses chosen are those amounts with which a majority of rats shed bloody tears from both eyes detectable with the naked eyes without the aid of filter paper. They are expressed as the weights of acetylcholine iodide on the basis of 100 g body weight of the rats. The results of these experiments are given in Table I, in which the value for intraperitoneal dosage without eserine represents an average, as one naturally expects a wider variation under this condition. Freud states the normal effective dose (intraperitoneal) in rats of 100-120 g to be =6 mg, which we presume to be of the iodide and to mean that the minimal dosages are around 6 mg.

The fact that the rat is more sensitive to intravenous injection

[.] Thanks are due to Dr. A. R. Sabin for his kind suggestions as to technic of intravenous injection in rats.

In early postnatal life the peaks of the loops are still covered by this type of epithelium, while in the second year the histological appearance is similar to that of the adults.

Further studies are needed to prove the validity of this observation and to further elucidate its mechanism.

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Chromodacryorrhea, a New Criterion for Biological Assay of Acetylcholine.

SHIRO TASHIRO, CARL C. SMITH,* ELIZABETH BADGER AND EDWARD KEZUR.

From the Department of Biochemistry, College of Medicine, The University of Cincinnati.

The peculiar phenomenon of the shedding of bloody tears by rats was reported in connection with the studies of dacryorrhetin, a compound prepared from muscle.^{1, 2} This phenomenon is so unique and easily distinguishable from ordinary lacrimation that the term, chromodacryorrhea, is proposed for it. It has served as a very convenient criterion for the biological assay of dacryorrhetin.

When Selye³ published a paper in which he quotes Freud's observation⁴ that acetylcholine causes rats to shed tears tinged red by blood,[†] one of us (T) examined chromodacryorrhetic and other properties of acetylcholine to see if dacryorrhetin could be in reality acetylcholine ⁵ The results of these investigations showed that these two compounds are not identical and at the same time suggested a possibility of using chromodacryorrhea as a new criterion for a biological assay of acetylcholine We have thus determined how small amounts of acetylcholine can be detected accurately by this criterion under different conditions

^{*} Charles Arnold Iglauer Fellow in Biochemistry

¹ Tashiro, Shiro, and Stix, Helen, Biol Bull, 1935, 64, 327

² Tashiro, Shiro, Proc Am Soc Biochem, 1937, 8, Nevin

³ Selye, Hans, Canadian Med Assn J, 1937, 36, 200

⁴ Freud, J., Acta Brevia Neerl , 1933, 3, 159

[†] Although no casual observer would question the presence of blood in tears, and it gives a positive benzidine reaction and its brinds are much like those of oryhemoglobin when examined with a hand spectroscope, yet Tashiro and Badger have evidence that the red pigment in the bloody tears is not oxyhemoglobin

⁵ Tashiro, Shiro, Kongressbericht II des XVI Internat Physiologenkongress 1938, 46.

Individual Variation of Rats. One should remember that occasionally he will find a rat which does not respond to dosages several times as large as the minimal effective dose. These variations must be due to the abnormal condition of Harder's glands, as one occasionally finds one or both glands lacking in some rats. There will be also a slight variation around the minimal dosage. Thus, if a large number of eserinized rats are injected intravenously with 0.2 7 acetylcholine iodide, there will be some which fail to shed bloody tears, although usually all will shed them with 0.3 7 dose. This variation is not due to a difference in sex or age. It is not probably due to variation in the esterase activity since a similar variation does occur when examined with the minimal dose of another choline derivative which is not destroyed by esterase. It is most likely due to a physiological condition of the glands, in which a slight change might be sufficient to produce a different response to the minimal dose. In any event, by selecting the minimum dosages in which the majority of rats shed bloody tears, this variation can be ignored.

Specificity of This Test. Chromodacryorrhea is not a specific test for acetylcholine any more than other methods of biological assay of acetylcholine. According to Freud, 0.5-1 mg of pilocarpine gives the reaction, but 20-80 mg of choline have no effect. We also find that choline and eserine do not produce chromodacryorrhea, although we noticed a few cases in which the animals shed the bloody tears during their death struggle following injection of very high toxic dosages of these compounds. Such rare cases will not interfere with the assay, for a mere dilution will eliminate the reaction. A powerful chromodacryorrhetic action of dacryorrhetin, although prepared from muscle, should not interfere with this test in a tissue analysis for acetylcholine, as this substance exists in the body as prodacryorrhetin which has no chromodacryorrhetic action. If necessary, one can easily distinguish dacryorrhetin from acetyl-choline by comparing the effective doses with and without eserine, as the action of the former will not be appreciably enhanced by eserine.

Summary. 1. By using chromodacryorrhea, the phenomenon of the shedding of bloody tears by rats, as a criterion, acetylcholine can be detected in as small amount as $0.2 \, \gamma$. 2. Since there are 4 ranges of minimal dosages detectable with this phenomenon, from 2 mg to $0.2 \, \gamma$, depending on the mode of injection and treatment, this same criterion can be used for a wide range of concentration of acetylcholine with accuracy.

TABLE I.

Minimal Chromodacryorrhetic Dosages of Acetylcholine Iodide, Calculated on the

Basis of 100 g Body Weight of Rats.

Mode of injection	Without eserine	With escrinized rats
Intraperitoneal	2,000	50.0
Intravenous	10-15	0.2

without eserine than to the intraperitoneal with eserine, and the fact that the eserinization increases sensitivity only about 50 times (10-15 γ to 0.2 γ) in the case of intravenous injection are rather interesting in view of the fact that one of us (S)⁶ found an exceedingly small amount of the esterase in the serum of rats as compared to human serum.

The Quantitative Assay. These minimum effective dosages can be used for the basis of the quantitative assay of acetylcholine, if less than 1 cc of the solution contains more than 0.2 y. Without eserinization, the intravenous injection gives fairly uniform results, but the results with intraperitoneal injection without eserine should be considered preliminary and approximate. The latter will be found useful when the available sample is large or highly concentrated such as in the study of acetylcholine synthesis and it will be exceedingly accurate for the estimation of other choline derivatives in which chromodacryorrhea is not enhanced by eserinization.

In either event, the quantitative assay of an unknown is done by determining the minimum positive and the maximum negative doses, accuracy depending upon the range between these two doses. For the actual analysis, a number of rats are weighed and about five rats are eserinized at the same time. Usually 0.1 cc of the solution per 100 g body weight is injected intraperitoneally. If the reaction is positive, a smaller amount or a diruted solution is similarly injected. If negative, the maximum injectable amount of the original solution should be injected intravenously to see if a measurable amount of acetylcholine is present. If positive a smaller amount or a diluted solution is injected. The cc per 100 g body weight with which the majority of rats give the reaction contain the minimum effective Thus the concentration per cc of the original unknown solution will be: for intraperitoneal injection, 50 $\gamma \div cc$ per 100 g body weight containing the minimum effective dose; and for intravenous injection. 0.2γ – cc per 100 g body weight. If the original solution is diluted, the value, of course, should be multiplied by the factor.

⁶ Smith, Carl C., unpublished data.

ceased and the placenta had separated. Hemoglobin was determined in grams percent with a calibrated Hellige-Sahli instrument. Red blood corpuscle counts were made with a Spencer hemocytometer (N.B.S.). Reticulocyte counts were obtained in 4 infants whose cords had been clamped immediately and in 4 in which clamping had been delayed.

Complete data will be published in a future article. The umbilical cord blood at the end of gestation in both series contained about 15.7 g of hemoglobin per 100 cc and about 4.5 millions of red corpuscles per cmm. Within a brief period of time after birthaveraging less than an hour in our experiments-the amount of hemoglobin and number of corpuscles in the newborn's blood increased markedly. Values rose to 21.3 g % Hb and 5.93 millions R.B.C. in those infants allowed to retrieve their placental blood. In those deprived of the placental blood by immediate clamping of the umbilical cord, the values rose only to 18.9 g % and 5.57 millions R.B.C. in the same interval. The amount of hemoglobin and number of corpuscles increased further during the course of the first postnatal day (22.5 g % Hb and 6.22 million R.B.C.) in the infants receiving their normal share of placental blood, but not in those deprived of it. The former group maintained higher values throughout the period under investigation.

One difference between the two experimental groups manifested itself on about the fourth day of life. Hemoglobin reached a peak in both series at that time, but those infants deprived of placental blood at birth exhibited an increase in hemoglobin, amounting to 1.4 g % above the one day average; while the other group showed a rise of only 0.5 g %. In neither series did the red corpuscle count rise.

Reticulocytes reached a peak at one day after birth. In a group of 4 infants whose cords were clamped immediately, this amounted to 8.3 % as compared with 4.7% in the 4 infants of the other group.

Our experiments have demonstrated that failure to allow the placental blood to return in large measure to the infant at the time of delivery is equivalent to submitting the newborn to a hemorrhage. Acceleration of hemopoiesis appears to occur in an attempt to make up this loss. It can not be doubted that a drain is placed upon the infant's iron reserve, and at this time in life it can ill afford such a loss, for it must get along with what iron it has in its body at birth until the nursing period is passed.

Our experiments may help to reconcile a persistent disagreement in the literature regarding hemoglobin and corpuscular values in

11561 P

Effect of Depriving Newborn of Placental Blood upon Early Postnatal Blood Picture.

Q. B. DeMarsh, W. F. Windle* and H. L. Alt.†

From the Anatomical Laboratories, Northwestern University Medical School, Chicago.

Within the last few years, the collection of postpartum placental blood for "blood banks" has been strongly advocated not only in Russia1 but also in Canada2 and in this country.3,4,5 It has been pointed out that this is an inexhaustible and "lucrative" source of blood, satisfactory for transfusion purposes, and that its collection has no deleterious effect upon the mother. Possible effects upon the child have been disregarded because it has already become a rather common obstetrical practice to clamp the umbilical cord promptly at birth in spite of the fact that most of the placental blood normally drains into the body of the infant within a few minutes when the umbilical cord is not clamped immediately after delivery.6 When the cord is clamped immediately, the infant is deprived of an alarming proportion of its total blood volume and usable iron at the very beginning of extra-uterine life. We have obtained proof that this blood-letting at birth affects the blood picture of the newborn significantly. The practice should be strongly condemned.

We have determined the amount of hemoglobin and number of red blood corpuscles in blood taken from the mother on the day of birth, in cord blood at birth, in blood from the newborn (heel) 15 to 75 minutes after birth, and from the infant at one, 3 to 4, and 6 to 7 days after birth in 2 series of patients. In one series of 25, the umbilical cord was clamped within 30 seconds after delivery; in another series of 29, clamping was delayed until pulsations had

^{*} Aided by a grant from the John and Mary R. Markle Foundation.

[†] Department of Medicine. This investigation was conducted at the Cook County Hospital, on the service of Dr. David S. Hillis, whose cooperation is greatly appreciated by the authors.

¹ Bruskin, Y. M., and Fackerova, P. S., Soviet Vrach. Zhur., 1936, No. 20, p. 1546 (cited by Gwynn and Alsever).

² Goodall, J. R., Anderson. L. O., Altimas, G. T., and McPhail, F. L., Sury., Gyn. and Obst., 1938, 66, 176.

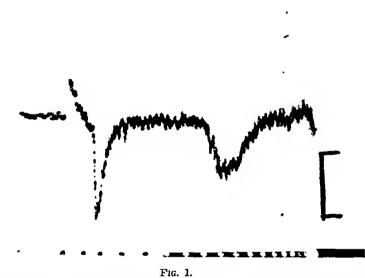
³ Grodberg, B. C., and Carey, E. L., New Eng. J. Med., 1938, 219, 471.

⁴ Gwynn, C. A., and Alsever, J. B., Am. J. Med. Sc., 1939, 198, 634.

⁵ Heyl, W. M., Am. J. Obst. and Gyn., 1940, 39, 679.

⁶ Haselhorst, G., and Allmeling, A., Z f. Geburtsh. n. Gynak., 1930, 98 103.

applied to the cerebral cortex by means of bipolar electrodes about 1 mm apart resting lightly on the pia. Monopolar recording was employed, the active electrode being a chlorided silver wire in light contact with the pia of the cerebellar cortex. Fig. 1 is a record of a cerebellar action potential obtained in this way. Following the shock artefact there is a surface positive wave having a latency of about 25 msec to the crest of the wave, and a second positive wave having a latency as long as 200 msec. The second wave by no means always accompanies the first, but the factors causing this wave have not been determined.



Cerebellar action potential recorded from Crus I, Lobulus ansiformus, as a result of a single electrical shock applied to the middle suprasylvian gyrus. The initial upward deflection is the shock artefact. Downward deflection indicates a surface positive potential. Time marks, 60 cycle; calibration mark, $200 \mu V$.

Stimulation of one cerebral cortical point may produce simultaneous potentials in as many as 14 distinct points on the cerebellar cortex. Considering the extensive foliation of this structure, it must be true that only a small fraction of the total surface was explored; the total number of points which yield potentials must be far greater than this. The most easily detected potentials are on the contralateral side, and in general for each of these potentials there is a smaller potential on the ipsilateral side at a point roughly symmetrical to it.

If the stimulus is well localized the cerebellar potentials may be very sharply localized; a point exhibiting a large potential may be man at birth. Some investigators have obtained results comparable with our determinations in cord blood and others have found higher values similar to ours in blood drawn from the infant after delivery. The exact source and time of collection of newborn blood have not always been stated. The difference which we have found between cord (venous) blood at the moment of birth and capillary blood from the infant less than an hour later is truly surprising but may be more apparent than real. A similar difference between venous and capillary blood has been reported in pernicious anemia but not in normal adults. It is possible that macrocytes in the blood of infants as well as of P.A. patients block some of the capillaries and thus effect a concentration of corpuscles.

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Cerebellar Action Potentials in Response to Stimulation of Cerebral Cortex.

HOWARD J. CURTIS. (Introduced by E. K. Marshall, Jr.)

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Medicine, Baltimore, Md.

The problem of functional localization in the cerebellum is one which has received considerable attention. Recent comparative anatomical studies¹ and ablation experiments² have supported a division of the cerebellum based on afferent fiber connections. Recently Dow³ has recorded action potentials in the cerebellum as a result of stimulating various afferent fiber tracts, and his results are in accord with Larsell's anatomical findings. The present work is an attempt to explore by the oscillographic method the projections of the cerebral cortex to the cerebellar cortex.

Methods and Results. Twelve cats, under barbiturate anesthesia, were used in this work. The method of stimulating and recording is described elsewhere. Single electrical shocks were

⁷ Waugh, T. R., Merchang, F. T., and Maughan, G. B., Am. J. Med. Sc., 1939, 198, 646.

s Duke, W. W., and Stoffer, D. D., Arch. Int. Med., 1922, 30, 94.

¹ Larsell, O., Arch. Neurol. Psychiat., 1937, 38, 580.

² Fulton, J. F., and Dow, R. S., Yale J. Biol. Med., 1937, 10, 89.

³ Dow, R. S., J. Neurophysiol., 1939, 2, 543.

⁴ Curtis, H. J., 1940, in preparation.

applied to the cerebral cortex by means of bipolar electrodes about 1 mm apart resting lightly on the pia. Monopolar recording was employed, the active electrode being a chlorided silver wire in light contact with the pia of the cerebellar cortex. Fig. 1 is a record of a cerebellar action potential obtained in this way. Following the shock artefact there is a surface positive wave having a latency of about 25 msec to the crest of the wave, and a second positive wave having a latency as long as 200 msec. The second wave by no means always accompanies the first, but the factors causing this wave have not been determined.

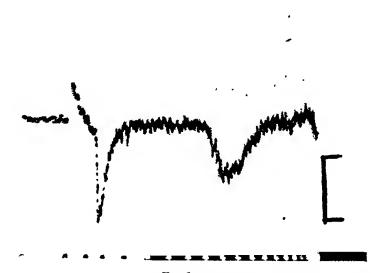


Fig. 1. Cerebellar action potential recorded from Crus I, Lobulus ansiformus, as a result of a single electrical shock applied to the middle suprasylvian gyrus. The initial upward deflection is the shock artefact. Downward deflection indicates a surface positive potential. Time marks, 60 cycle; calibration mark, $200~\mu V$.

Stimulation of one cerebral cortical point may produce simultaneous potentials in as many as 14 distinct points on the cerebellar cortex. Considering the extensive foliation of this structure, it must be true that only a small fraction of the total surface was explored; the total number of points which yield potentials must be far greater than this. The most easily detected potentials are on the contralateral side, and in general for each of these potentials there is a smaller potential on the ipsilateral side at a point roughly symmetrical to it.

If the stimulus is well localized the cerebellar potentials may be very sharply localized; a point exhibiting a large potential may be only 1 mm away from a point which shows no measurable potential. On the other hand, when the stimulating electrodes are moved the pattern of the potentials on the cerebellar cortex is changed, but the potential at any single point may remain almost unchanged while the stimulating electrodes are moved by as much as 2 cm. This phenomenon is not due to a spread of the stimulating current, since a displacement of the stimulating electrodes by only 1 mm will often very markedly change the pattern of the cerebellar response.

None of the areas in the cerebral cortex which have been explored has failed to produce at least one potential in the cerebellum, and these areas include the sigmoid gyrus, marginal gyrus, middle suprasylvian gyrus, and middle ectosylvian gyrus. It was found that with the exception of the declive and tuber vermis all of these areas project to the neocerebellum¹ and to the posterior part of the anterior lobe. Fig. 2 shows a dorsal view of the cerebellum; the cross hatching indicates regions from which potentials have been recorded, shading indicates regions explored without finding potentials, and the unshaded regions were not explored. There seem to be no anatomically distinct regions which can be said to be associated with particular areas of the cortex. In other words, any given region in the cross-hatched areas of Fig. 2 may receive im-

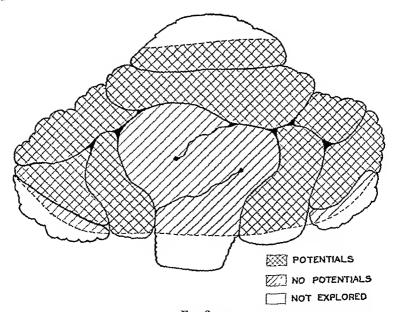


Fig. 2.

Diagram of dorsal view of cat's eerebellum showing regions from which potentials have been obtained as a result of stimulating the cerebral cortex.

pulses from any or all of the areas of the cerebral cortex which were explored.

The application of a small quantity of 0.3% picrotoxin solution to the surface of the pia over a small region exhibiting one of these potentials, radically changed the size and shape of the recorded potential. The initial surface positive wave was immediately followed by a large surface negative wave, giving the response a diphasic appearance. The effect seems to be quite similar to that observed in the cerebral cortex⁴ except that it is necessary to use more concentrated solutions in the case of the cerebellum to evoke the response, a fact which may explain why Dow⁵ failed to observe "strychnine spikes" in the cerebellum.

Discussion and Conclusions. These results appear to support and extend the ideas of functional localization in the cerebellum⁶ as opposed to those of anatomical localization.⁷ They indicate that there is no region in the neocerebellar cortex which can be said to be particularly related to any region of the cerebral cortex. Since the cerebellum has come to be known as an organ of synthesis and coordination, it would hardly seem strange (a) that many different functional areas in the cerebral cortex are connected with a single point in the cerebellum, and (b) that a single cerebral cortical point projects to a number of cerebellar foci. This is in good agreement with Dow's work³ in which he found that stimulation of spinal nerves produced a pattern of potentials in the anterior lobe of the cerebellum which changed very little when nerves from different parts of the body were stimulated.

The fact that most of the individual cerebellar potentials obtained in the present study are very sharply localized perhaps throws some doubt on the concept of mass function of the cerebellum, at least as far as the afferent connections are concerned. The results indicate that a relatively small number of cerebral cortical efferents are capable of exciting a large number of small isolated cerebellar units. Thus it appears probable that a single cortical efferent makes synaptic connections with several cerebellar afferents in the pontine nucleii. The occurrence of multiple potentials over the surface of the cerebellar cortex cannot be due to spread of excitation there, since there is no very appreciable difference in latency between the potentials recorded from the different points which form any one pattern. The distribution of potentials shown in the map of Fig. 2 is, in

Dow, R. S., J. Physiol., 1938, 94, 67.

⁶ Sherington, C. S., in Schäfer, Textbook of Physiology, 1900, 2, 884.

⁷ Bolk, L., Das Cerebellum der Saugetiere. 1906, Harlem, Bohn.

only 1 mm away from a point which shows no measurable potential. On the other hand, when the stimulating electrodes are moved the pattern of the potentials on the cerebellar cortex is changed, but the potential at any single point may remain almost unchanged while the stimulating electrodes are moved by as much as 2 cm. This phenomenon is not due to a spread of the stimulating current, since a displacement of the stimulating electrodes by only 1 mm will often very markedly change the pattern of the cerebellar response.

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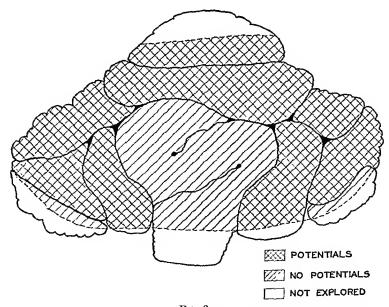


Fig. 2.

Diagram of dorsal view of cat's eerebellum showing regions from which potentials have been obtained as a result of stimulating the cerebral cortex.

SECRETARY'S REPORT

April 1, 1939—March 31, 1940

The annual meeting of the Council took place Maich 15, 1940 at New Orleans. Members present: Doctors Dorsy, Gasser, Leake, Meek, Smith, Soskin and Thienes; by proxy, Doctor Adolph (for Dr. Hodge), Dr. Green (for Dr. Ingram), Dr. Myers (for Dr. Hayman), Dr. Roe (for Dr Leese), and Dr. Tainter (for Dr. Meyer).

Membership The Council elected 103 applicants for membership, previously approved manimously by the National Membership Committee. The Council elected 5 applicants not unanimously approved by the Membership Committee. The Council approved the recommendation of the National Membership Committee that action on 4 applications be deferred pending further publication. (The National Membership Committee by a majority voted to defer action on 23 applications pending further publication.)

The Council manimously voted that only those applications for membership received by the Secretary-Treasurer by December 15 shall be forwarded to the National Membership Committee for report to the Council at its annual meeting

nual meeting

The following resignations were accepted with regret: Doctors M L. Anson, I. S Falk, F H Falls, F B. Flinn, H. J. Fry, R. Guy, W. W. Hamburger, J Markowitz, F. L Meleney, L. F. Rettger, H. B. Richardson, H. Sommer, A Steindler and W. G Young.

Five members were declared in arrears and dropped from the membership list

Two members in China were given an extension of time to clear their accounts

Doctors Guy N. Calkins and P. A. Levene were declared emcritus members

Ballot The Council unanimously voted that the ballot with nominees for national offices shall be distributed to members within 30 days after the annual meeting of the Council

Honorary Members The Council approved the recommendation of the Committee that the Society discontinue the election of honorary members

Finances The Treasurer reported a deficit of \$2,319 for the 11 months' period Λ pril 1, 1939 to March 1, 1940, or about \$2,500 for the fiscal year (Details are given in the Treasurer's annual report). This deficit was due primarily to the following:

- 1 Greater increase in size of Proceedings than had been anticipated
- 2 The low charge for excess space, 20 percent of cost.

3 The low membership dues.

This deficit will be paid from accumulated profits of preceding years, viz, Sniplus Fund

To meet the expected deficit for the coming year, the Conneil approved the following changes:

- 1 Increase the charge for excess space from 20 to 40 percent of cost,
- 2 Increase subscription price (to non-members) from \$6.00 to \$7.50 a year
- 3 Use interest of Surplus Fund.

general, what one would expect from the known cerebellar afferent connections assuming that all cerebro-cerebellar connections are effected by synapses in the pontine nucleii. Dow,³ by directly stimulating the pons, obtained potentials not only in all areas from which potentials were recorded in the present work, but also in the declive and tuber vermis, pyramis, and paraflocculus. The fact that no potentials have been obtained from the declive and tuber vermis on cerebral cortical stimulation is interesting in view of the fact that Larsell¹ includes this part of the organ in the neocerebellum. It should be emphasized, however, that one must interpret the absence of potentials with extreme caution.

District of Columbia

Chairman: C. E. Lecse. Secretary: D. B. Jones. Members: 42.

Meetings: U. S. Public Health Service, December 7, 1939

Illinois

Chairman: T. E. Boyd. Secretary: A. Weil. Members: 139

Meetings: University of Chicago October 24 1939

University of Illinois Medical School, December 12, 1939

Northwestern University, January 23, 1940 University of Chicago, April 9, 1940

University of Chicago, April 9, 1940 Northwestern University, May 21, 1940

Iowa

Chairman: E. D. Plass. Secretary: T. L. Jahn. Members: 39

Meetings: State University of Iowa, February 14, 1940 April 26, 1940

Minnesota

Chairman: F. H. Scott. Sccretary: F. H. Scott. Members: 51

Meetings: University of Minnesota, October 18, 1939

December 20, 1939 February 21, 1940 March 20, 1940 April 17, 1940 May 15, 1940

Missouri

Chairman: A. B. Hertzman. Secretary: H. L. White. Members: 55 Meetings: St. Louis University Medical School, October 11, 1939

Washington University Medical School, December 13, 1939 St. Louis University Medical School, February 14, 1940

Washington University Medical School, April 10, 1940 St. Louis University Medical School, May 8, 1940

New York

Chairman: J. C. Hinsey. Secretary: I. Greenwald. Members: 453

Meetings: New York Academy of Medicine, October 18, 1939

New York Medical College, November 22, 1939 Cornell University Medical College, January 24, 1940

New York University, February 28, 1940 Rockefeller Institute, April 24, 1940

College of Physicians and Surgeons, May 22, 1940

Pacific Coast

Chairman: A. W. Meyer. Secretary: C. Weiss. Members: 101

Meetings: University of California, October 21, 1939

Stanford University Medical School, December 8, 1939 University of California Hospital, February 7, 1940

Mount Zion Hospital, March 8, 1940

By-Laws. Suggested changes in the by-laws were proposed. It was decided to request the President to appoint a committee of three in or near New York to consult with the Secretary-Treasurer to propose changes in the by-laws.

Editors. The following were elected to the Editorial Board:

C. A. Elvehjem, in biochemistry of vitamins

R. J. Dubos, in physiology and metabolism of bacteria

E. M. K. Geiling, in pharmacology

E. M. Marshall, Jr., in pharmacology.

National Membership Committee. This committee held its annual meeting March 15, 1940, to reconsider those applications for membership which had not been unanimously approved by them. Their findings were reported to the Council, (see above). A further effort was made to define more precisely what constitutes eligibility to membership.

PAST OFFICERS

Date	President	Vice-President	Secretary	Treasurer
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	33° 33	11 11
1905-06	E. B. Wilson	E. K. Dunham	21 21	" "
1906-07	S. Flexner	E. K. Dunham	" "	" "
1907-08	S. Flexner	T. H. Morgan	" "	27 17
1908-09	F. S. Lee	T. H. Morgan	11 11	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie)) 11
1910-11	T. H. Morgan	W. J. Gies)1 ji	» 11
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	" "
1912-13	J. Ewing	P. A. Levene	» »	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackon	" "
1914-15	G. Lusk	W. J. Gies	11 11	J. R. Murlin
1915-16	G. Lusk	G. N. Calkins		Jackson
1916-17	J. Loeb	W. J. Gies	**	11
1917-19	W. J. Gies	J. Auer	*11	97
1919-21	G. N. Calkins	G. B. Wallace	**	,,
1921-23	G. B. Wallace	J. W. Jobling	n	"
1923-24	H. C. Jackson	J. W. Jobling	y. c.	Myers
1924-25	H. C. Jackson	J. W. Jobling	А. J.	Goldforb
1925-27	J. W. Jobling	S. R. Benedict	"	"
1927-29	S. R. Benedict	P. Rous	"	"
1929-30	P. Rous	D. Marine	"	"
1930-31	P. Rous	D. J. Edwards	"	"
1931-32	D. J. Edwards	A. R. Dochez	,,	"
1932-34	A. R. Dochez	E. L. Opie	,,	,,
1934-36	E. L. Opie	P. E. Smith	"	"
1936-37	P. E. Smith	E. F. DuBois	,,	,,
1937-39	H. S. Gasser	J. T. Wearn	"	"
1939-40	J. T. Wearn	C. D. Leake	,,	

SECTIONAL MEETINGS AND MEMBERSHIP

Cleveland, Ohio

Chairman: H. Feil. Secretary: W. Hambourger. Members: 42.

Meetings: Western Reserve University, October 13, 1939

November 10, 1939 December 8, 1939 January 12, 1940 February 9, 1940 March 8, 1940 April 12, 1940

TREASURER'S REPORT

April 1, 1939-April 1, 1940

Balance on hand, April 1, 1939.	••••	\$ 7,433.16
Income		
Dues	\$ 5,959.25	
Reprints	4,120.48	
Space	1,244.45	
Cuts	789.12	
Changes	66.39	
Subscriptions	3,814.48	
Back Numbers		
Interest from special accounts		
Miscellaneous		
httsechalicous	20.02	\$16,282.97
		φ10,203.01
Total Cash Available, April 1, 1939-April 1, 1940	·	\$23,716.13
Disbursements		
Printing	\$10,958.70	
Reprints		
Cuts		
		\$15,848.66
		410,010.00
Office Cumpling Destant Telephone	000 17	
Office Supplies, Postage, Telephone		
Salary		
Storage and Insurance		
Miscellaneous		
		\$ 3,111.05
		¢10 050 71
Cash balance, April 1, 1940		\$18,959.71
2, 2020	•	4,756.42
		202 71 6 1 2
		\$23,716.13
Summary		
Income (net)	\$16,282.97	7
Disbursements (net)	. 18.959.71	1
		-
Defici	it \$ 2,676.7-	1
Interest from Surplus and Endowment Funds t	Λ ΄	-
be used	1,161.5	Ī
		_
Net Defic	it \$ 1,515.2	3
Bills receivable—\$889.98	7 3,030.20	•
·		
Bills payable—None.		

Peiping, China

Chairman: A. B. Fortuyn. Secretary: F. T. Chu. Members: 30 Meetings: Peiping Union Medical College, October 25, 1939 February 7, 1940

May 9, 1940

Southern

Chairman: H. S. Mayerson. Secretary: R. Ashman. Members: 41

Meetings: Tulanc University, November 3, 1939
January 26, 1940
Louisiana State University, May 3, 1940

Southern California

Chairman: E. Bogen Sceletary: M. S. Dunn. Members: 40 Meetings: University of Southern California, October 12, 1939

Los Angeles County General Hospital, November 30, 1940

Monterey, Calif., December 20, 1940

Scripps Institution of Oceanography, March 2, 1940 University of California, Los Angeles, May 2, 1940

Western New York

Chairman: E. F. Adolph. Secretary: H. C. Hodge. Members: 62

Meetings: Syraeuse University, October 14, 1939

University of Buffalo, December 9, 1939 University of Rochester, February 17, 1940

Cornell University, May 18, 1940

Wisconsin

Chamman: W. E. Sullivan. Secretary: O. O. Meyer. Members: 41

MEMBERSHIP

Members, March 31, 1939			1488
Elected during year			23
Total .			1511
Resignations			25
Deaths			11
Arrears			10 46
Total Membership, March 31, 1940			1465
••	1930	1940	
Membership	1026	1465	
Subscriptions, March 31, 1940			608

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: Doctors H. V. Atkinson, A. H. W. Caulfeild, C. B. Coulter, D. M. Cowie, G. E. Cullen, H. W. Cushing, W. T. Dawson, F. P. Gay, R. H. Jaffe, F. S. Lee, H. A. McCordock, E. B. McKinley, D. Perla, O. H. Plant, and I. C. Wen.

Dr. O. H. Plant was a member of the Editorial Board from 1936 to the fall of 1939. With his death the Society lost a genial, devoted, considerate and eminently fair member of the Board. He aided materially in developing an ever better standard for the PROCEEDINGS

MEMBERS' LIST

HONORARY MEMBERS

Flexner, Simon Howell, William H Poiter, William Richet, Chailes Von Muller, Friedrich Rockefeller Inst.
Johns Hopkins Univ.
Harvard Univ.
Paris, France
Munich, Germany

MEMBERS

Abramson, D 1 Abram-on, H. A Abt, Arthur F Adam-, A Elizabeth Adam-, William E Addis, Thomas Adler-berg, D Adolph, E F Adolph, W H Alexander, Harry L Allen, Bennet M Allen, Edgar Allen, William F Alles, G. A Almquist, H J Alt. Howard L Althausen, T L Alt-chule, M D Altshuler, S S Alvarez, Walter C Alving, A S Amberz, Samuel Amherson, W R Amoss, Harold L Anderson, Dorothy H. Anderson, H H Ander-on, John E Anderson, John F Anderson, Rudolph J Anderson, William E. Andrews, Edmund An-bacher, Stetan Antopol, William Appealy, Frank L Armstrong, Charles

May In-t. Med. Research, Cincinnati Coll. Physicians and Surgeons, New York Northwestern Univ. Mount Holyoke Coll Univ. of Chicago Stanford Univ. Med. Beth Israel Hosp, N Y. Univ. of Roche-ter Med. Perping, China Washington Univ Univ of Calif., L A Yale Univ Univ. of Oregon San Marino, Calif Univ of Calif. Northwestern Univ. Med Univ. of Calif. Med Beth I-rael Hosp, Boston Wayne Univ. Med Mayo Clime Univ. of Chicago Mayo Clinic Univ. of Maryland Med Rockefeller In-t Coll. of Phys. and Surg , N Y Perping Union Med Coll Univ of Minn E. R Squibb & Son Yale Univ Springheld, Mass Bloomington, Ill Squibb Inst, New Brunswick, N. J. Beth Israel Hosp , Newark, N J. Med. Coll of Va. National Inst of Health, Washington

FUNDS

Endowment Fund

April 1, 1939.	\$17.304.68	
Interest to April 1, 1940		
		\$17,965.95
Invested in New York Title and Mortgage Co		
Title Guarantee and Trust Co	2,000.00	
Lawyers Mortgage Co		
Bowery Savings Bank	2,989.46	
United States Savings Bonds	1,875.00	
Industrial Bonds	3,721.49	
		\$17,965.95
Surplus Fund		
April 1, 1939	\$10,924.16	
Interest to April 1, 1940.		
·		\$11,424.40
Invested in Title Guarantee and Trust Co	\$ 2,850.50	,
Harlem Savings Bank	824.71	
United States Savings Bonds	1,875.00	
Industrial Bonds	5,874.19	
-		\$11,424.40
Life Membership Fund		
Invested in Railroad Federal Savings and Loan		\$ 75.00
invested in italifold rederal pavings and Loan		4 10.00

Auditors' Report

We the undersigned have this day examined the Treasurer's report and find it to agree with the books of the Society. We believe that the records of the financial transactions are accurate and in good order.

We reiterate the request of previous committees that a certified public accountant should be engaged to make periodic audits of the Treasurer's records for the protection of the Secretary-Treasurer and the members of the Auditing Committee.

(Signed) ALEXANDER B. GUTMAN HOMER W. SMITH WILLIAM S. TILLETT

May 3, 1940.

MEMBERS' LIST

HONORARY MEMBERS

Flexner, Simon	Rockefeller Inst.
Howell, William H.	Johns Hopkins Univ.
Porter, William	Harvard Univ.
Richet, Charles	Paris, France
Von Muller, Friedrich	Munich, Germany

MEMBERS

Δ bramson, D. I	May Inst. Med. Research, Cincinnati
Abramson, H. ACo	II. Physicians and Surgeons, New York
Abt, Arthur F.	
Adams, A. Elizabeth	
Adams, William E.	Univ. of Chicago
Addis, Thomas	Stanford Univ. Med.
Adlersberg, D.	Beth Israel Hosp., N. Y.
	Univ. of Rochester Med.
Adolph, W. H.	Peiping, China
Alexander, Harry L.	
Allen, Bennet M.	Univ. of Calif., L. A.
Allen, Edgar	Yale Univ.
Allen, William F	Univ. of Oregon
Alles, G. A.	San Marino, Calif.
Almquist, H. J.	Univ. of Calif.
Alt, Howard L.	
Althausen, T. L.	Univ. of Calif. Med.
Altschule, M. D.	Beth Israel Hosp., Boston
Altshuler, S. S.	
Alvarez, Walter C	Mayo Clinic
Alving, A. S.	Univ. of Chicago
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	Univ. of Maryland Med
Amoss, Harold L	Rockefeller Inst.
Anderson, Dorothy H	Coll. of Phys. and Surg., N. Y.
Anderson, H. H.	Peiping Union Mcd. Coll.
Anderson, John E	Univ. of Minn.
Anderson, John F	E. R. Squibb & Son
Anderson, Rudolph J	Yale Univ.
Anderson, William E	Springfield, Mass.
Andrews, Edmund	Bloomington, Ill.
Ansbacher, Stefan	Squibb Inst., New Brunswick, N. J.
Amopol, William	Beth Israel Hosp., Newark, N. J.
Apperly, Frank L.	Med, Coll. of Va.
Armstrong, Charles	National Inst. of Health, Washington

Arnold, Lloyd	Univ. of Ill.
Aronson, J. D.	Henry Phipps Inst.
Asdell, S. A.	Cornell Univ.
Asher, Lcon	Berne, Switzerlaud
Ashman, Richard	Louisiana State Univ.
Asmundson, V. S.	Univ. of Calif.
Atchley, D. W	Presbyterian Hosp., N. Y. C.
Atwell, Wayne J	Univ. of Buffalo
Aub, Joseph C	Huntington Memorial Hosp., Boston
Auer, John	St. Louis Univ.
Austin, J. Harold	Univ. of Pa.
Avery, B. F.	Am. Univ. of Beirut
Avery, O. T.	
Avery, Roy C.	Vanderbilt Univ
Ayeoek, W. L.	Harvard Ved.
22,000, 11. 24	till tulu sucu-
Rabkin, Boris P	MeGill Univ.
Bachem, Albert	Univ. of Ill. Med. Coll.
Bachr, George	Mt. Sinai Hosp., N. Y. C.
Bagg, Halsey J	Memorial Hosp., N. Y. C.
Bahrs, Alice M.	Portland, Ore.
Bailey, Cameron V.	N V Post-Graduate Med.
Baitsell, George A	Yale Univ.
Baker, Lillian E.	Rockefeller Inst.
Bakwin, Harry	N V Univ Med Coll.
Baldwin, Francis M.	Univ of S Calif.
Ball, G. H.	Univ of Calif L. A.
Ball, H. A.	San Diago Calif
Balls, A. K.	II S Dent of Agr.
Banting, Frederick G.	Univ of Toronto
Barach, Alvan L	Call of Phys and Surgeons, N. Y.
Barber, W. Howard	New York Univ. Med.
Barbour, Henry G	Vale Univ.
Barer, Adelaide P	State Univ of Iowa
Barlow, O. W	Rengselaer N. Y.
Barnett, George D	Stanford Univ.
Barr, David P	Washington Univ.
Barron, E. S. G	Univ of Chicago
Barth, L. G.	Columbia Univ.
Bartley, S. H	Washington Univ.
Bass, Charles	Tulane Univ
Bast, T. H	Univ of Wisconsin
Bates, R. W	Carnegie Inst. of Washington
Bauer, J. H	Rockefeller Inst.
Bauman, Louis	Preshyterian Hosp., N. Y. C.
Baumann, E. J	Montefiore Hosp., N. Y. C.
Denne J Perev	Stanford Univ.
Towar C	Yale Univ.
	Univ. of Pa.
Bean, John W	Univ. of Mich.
Bean, John W	

	T 71 . T
Beard, H. H	Louisiana State Univ.
Beard, J. W	Duke Univ.
Reard P J	Stanford Univ.
Beck, Claude S	Western Reserve Univ.
Reeker E R	Iowa State Coll.
Beckman, Harry	Marquette Univ. Med.
Beckwith, T. D.	Univ. of Calif., L. A.
Behre, Jeannette A	Cornell Univ. Med. Coll.
Belding, David L.	Boston Univ.
Rander M B	Mt. Sinai Hosp., N. Y.
Bengston Ida A	National Inst. of Health, Washington
Rayor B V	Columbia Univ.
Barg C P	State Univ. of Iowa
Barg William V	N. Y. City
Powerin Olaf	Univ. of Ill.
Dergeiii, Olai	Rockefeller Inst.
Derginann, Max	Lenox Hill Hosp., N. Y. City
Bernnard, Adolph	This of Mich
Bernthal, T. G	
Berry, George P	Univ. of Rochester Med.
Beutner, R	Hahnemann Med. Coll., Philadelphia
Bierman, W	Mt. Sinai Hosp., N. Y.
Bieter, Raymond N	Univ. of Minu.
Bills, C. E	Mead, Johnson and Co., Evansville, Ind.
Bing, Franklin C	Am. Med. Assn., Chicago
Binger, Carl A. L.	Rockefeller Inst., N. Y.
Birkhaug, Konrad E	Geofysisk Inst., Bergen, Norway
Bishop, George H	Webster Groves, Mo.
	Univ. of Calif.
Blackfan, K. D	
Blair, John E.	
Blake, F. G	Vale Univ
Blakeslee, Albert FSta.	for Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt Univ Med
Blatherwick, Norman R	Metropolitan Life Insurance Co., N. V. City
Blau, Nathan F	Cornell Univ. Med. Coll.
Blinks, L. R.	Stauford Univ.
Bliss, Siduev	Tulane Liniv
Bloch, Robert G	Univ. of Chieago
Block, Richard J.	
Bloom, William	Univ. of Chicago
Bloomfield, A. L.	Stanford Univ. Med.
Bloor, W. R.	Univ. of Rochester
Blount, R. F.	Univ. of Minn.
Blum, Harold F.	Washington, D. C.
Blumberg, Harold	Johns Hopkins Univ.
Blungart, H. L.	Beth Israel Hosp., Boston
Bock, Joseph C	Beth Israel Hosp., Boston Marquette Univ.
Bodansky, A.	Hosp. for Joint Diseases, N. Y.
Radaushy Waran	
	John Casle II Other m
Budansky, Meyer	John Sealy Hosp., Galveston, Texas New York Univ

Bodine, J. H	State Univ. of Iowa
Bogen, Emil	Olive View, Calif.
Boissevain, Charles H	Colorado Coll.
Bollman, Jesse L	Mayo Clinic
Bonner, James	Calif. Inst. of Technology
	Univ. of Chicago
Boothby, Walter M.	Kahler Hosp., Rochester, Minn.
Boots Rainh H	Presbyterian Hosp., N. Y.
Rorenck Harry	Calif. Inst. of Technology
	Buffalo Gen. Hosp.
	Queens Univ., Canada
	Loyola Univ.
Doyll, Theo. 12	Univ. of Minn. Med.
Boxler, Emil	Ohio State Univ.
Bradford, William L	Univ. of Rochester
Bradley, H. C	Univ. of Wiseonsin
Brand, Erwin	
Branham, Sara E	National Inst. of Health, Washington
Brewer, George	Univ. of Pa-
Brewer, Robert K.	Syraense Univ.
Briggs, A. P.	Univ. of Georgia
Bronfenbrenner, J	Washington Univ.
Bronk, D. W	Univ. of Pa.
Brooks, Clyde	Louisiana State Univ.
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Hale, William M	Ctate Time of Town
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_ ` + 13	Ohio State Univ.
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3C -1 41fmad D	TOCKETENET INSE
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Moore Carl R	Univ. of Unicago
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a cura i Ti a calcilia R	Jefferson Med. Coll.
- la D	Hniv of W Oncario
Millet, John A. P.	

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Dannanhaimar A V	Coll. of Pays. and Durg., N. 1.
The state of the s	narvaru uuv
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C	Here of Pleasinger
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Interior's	

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Smith, Millard	Boston, Mass.
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Smith, Philip E	Columbia Univ
Smithburn, K. C.	TT 1 TO A C.C.
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Soffer, Louis J	Mt Singi Hosp N V
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Somogyi, Michael	Jewish Hosp St Louis Mo
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Swett, Francis H	Duke Univ.
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Cyclonotyickov V P	Univ. of Georgia Med.
Syverton Jerome T.	Univ. of Rochester
Mainton Mannica I.	Stanford Univ.
Tainter, manife in an in a manife in a man	Univ. of Ill.
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Tashiro, Shiro	Univ. of Wise
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W W	Univ. of Chicago
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Sweet, J. Edwin	Cornell Univ. Med. Coll.
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	Dulto Univ
Swett, Francis H	Duke Univ.
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Tannar Brad W	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
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15170

Oral Use of Penicillin in Treatment of Experimental Erysipelothrix rhusiopathiae Infection in Mice.

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The development of an effective treatment for swine erysipelas becomes of considerable importance because of the increasing prevalence of this disease in the United States and in foreign countries. The present study was therefore undertaken to evaluate the use of penicillin in the control of experimental infec-Heilman and Herrell1 have reported successful treatment of infected mice by frequent injections of penicillin, totaling 1000 units per day per mouse, over a 7-day period. Since simplicity of treatment often becomes a prime factor in the field of animal disease control, we were particularly interested in the possibi ity of oral administration rather than the injection method.

Procedures. Mice: Test animals throughout were 16 to 18 g mice. For the major por-

tion of tests, Rockland female mice were used.[†] At one stage it was necessary to substitute a Dilute Brown Agouti, tumor-susceptible strain due to scarcity of the Rocklands. Comparative tests using the 2 strains showed no difference in response.

Penicillin-in-feed: In early trials penicillin was suspended in corn oil in a concentration of 1000 units per cc of oil. This was then mixed with a cereal type feed to give a final concentration of either 100 or 1000 units of penicillin per gram of feed. In later tests, the dried penicillin was ground with dried feed in varying concentrations and fed with no protective coating of oil. Test mice were placed in individual cages and given weighed amounts of penicillin-containing food. After 24 hours, animals were challenged with an injection of 10 MLD of test culture. Feeding with treated material was continued for the

^{*} American Cyanamid Research Laboratories, Stamford, Connecticut.

¹ Heilman, F. R., and Herrell, W. E., Proc. Staff Meetings of the Mayo Clinic, 1944, 19, 340.

t Obtained from Rockland Farms, New City, N.Y.

TABLE I. Effectiveness of Penicillin in Feed.

Method of				
administration	10 units/g	100 units/g	1000 units/g	2500 umts/
Oil	0/10*	0/10	0/10	0/10
Dry	0/10	0/10	10/10	10/10
				20,20

^{*} Survivals/mice injected.

full time of the experiment. A like number of animals were fed a normal diet and served as control animals.

Penicillin-Drinking Water: Penicillin was diluted with tap water in concentrations ranging from 10 to 1000 units per cc of water. Fifty cc amounts of final dilution were supplied to each cage of 5 mice. This amount was enough for 48 hours in most instances. The penicillin-water was replaced every 48 hours with fresh material, regardless of any amount which might remain. In original trials, animals were given the treated drinking water for 24 hours to develop a blood level prior to challenging. In later experiments the challenge dose and beginning of treatment were simultaneous. In final trials, the challenge took place 24 or 48 hours prior to the beginning of treatment. The length of time for continuance of treatment varied from 24 hours to 10 days—the duration of tests.

Culture: The test culture used throughout was Erysipelothrix rlusiopathiæ, Lederle No. 358. This strain is used routinely for protection tests against anti-swine erysipelas serum and is of extremely high virulence for mice. Subcutaneous injection of 0.5 cc of a 10-8 dilution of culture will consistently kill 100% of unprotected mice, and a similar injection of a 10-9 dilution usually kills at least 2 out of 3 mice.

Results. Effectiveness of penicillin-in-feed: The oil-coated penicillin appeared to be very unpalatable and was eaten in smaller amounts than the control feed. For this reason, the mice failed to develop a protective blood and all animals died within 4 days fror date of challenge. When the dried ma was ground with the feed in a concentr of 1000 units per gram, or more, a satisfa level was obtained and 100% protection afforded, while all control animals died w 4 days of the date of challenge. (Ta shows sample experiment.)

Effectiveness of penicillin-in-water: It tion of 1000 units of penicillin per cc of a provided complete protection when begated advance of or simultaneously with the it tion of the challenge dose. When the contration was cut to 500 units per cc, the tective rate dropped to 20% of the test mals. Concentrations lower than 500 per cc were of no protective value even a supplied for 48 hours prior to the dat challenge. (Table II.)

Tests were then carried out to determine whether or not the 1000-unit concentration would be effective against more than the MLD challenge dose. Injections were refrom serial dilutions prepared to give 1000, and 10,000 MLD and treatment begun simultaneously. Complete protect was provided against even the highest nur of MLD used for challenging.

When challenge injections were made I to the beginning of treatment, results variable. Treatment begun 24 hours a challenge in some instances gave almost c plete protection, while in duplicate trials (20% survival resulted. After 48 hours his

TABLE II.
Effectiveness of Penicillin in Drinking Water.

Treatment begun	10 units/ec	100 units/cc	500 units/ce	1000 umts/ce	5000 units/ce	10,000 units/ee
at he before challenge	0/10	0/10	2/10 0/10	10/10 10/10	10/10 10/10	10/10 10/10
Simultaneously with challenge 24 hr after challenge				4/10 0/10	5/10 0/10	5/10 0/10
48 hr after challenge						

dosages, even up to 10,000 units per cc, were of no value and all animals died.

Discussion. The evidence presented here indicates that penicillin, with no protective coating or buffering materials, may be successfully administered to mice by mouth and, provided that sufficiently large quantities are given, protection is afforded against E. rhusiopathiæ. Since 50 cc of water containing 1000 units/cc was consumed by 5 mice in 48 hours, the effective average intake per mouse was 5000 units/day. This is approximately 5 times the amount used by Heilman and Herrell¹ in injecting the material. Penicillin is effective in the treatment of Erysipelothrix rhusiopathiæ infection if given early enough after exposure. Our results on this point are in accord with the findings of Heilman and Herrell¹ who showed protective action by the injection method of administration when begun 21 to 24 hours after challenge. Under our experimental conditions the effective time

interval was 24 hours or less, but in field conditions institution of therapy at such an early stage of infection may be unattainable. It would seem, therefore, that penicillin may be of considerable value only in the control of the outbreak of this infection in domestic animals. The ease of oral administration makes its use feasible on a far greater scale than would be possible where injections are necessary.

Summary. Penicillin is shown to be of value in the protection of mice against experimental Erysipelothrix rhusiopathiæ infection.

Oral administration is successful in the treatment of mice even without any protective action from oil coating, or buffers, provided it is instituted within 24 hours after exposure. The use of penicillin in drinking water is found to be as effective as is its addition to the feed. Oral administration compares favorably with parenteral injection although larger amounts are required.

15171

Preparation of Human Typing Sera by Iso-immunization of Human Donors with Group Specific Substances.

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observations.

Typing sera with extremely high agglutination titers have been prepared by Witebsky and his coworkers¹ in human donors following the intravenous injection of solutions containing minute amounts of group A and B specific substances. Wiener,² employing intramuscular injections of autoclaved saliva from group A and B individuals (secretors) instead of isolated group specific substances, confirmed Witebsky's observations. Pillemer et al.³ have

prepared as by-products of plasma fraction,

concentrated isohemagglutinin globulins that

Nine subjects, 3 belonging to each of the following groups, O, A, and B, received intravenously 0.5 cc of a solution of group A and B

were characterized not only by their high titer, but by their high avidity as well, *i.e.*, by the speed with which the serum agglutinated red cells. To the best of our knowledge, an investigation of the change in the avidity of typing sera following isoimmunization of human donors with group specific substances has not appeared in the literature. In the course of studies on blood group specific substances, we have had the opportunity to make such

¹ Witebsky, E., Klendshoj, N. C., and McNeil, C., Proc. Soc. Exp. Biol. and Med., 1944, 55, 167.

² Wiener, A. S., Soble, R., and Polivka, H., Proc. Soc. Exp. Biol. and Med., 1945, 58, 310.

³ Pillemer, L., Oncley, J. L., Melin, M., Elliot, J., and Hutchison, M. C., J. Clin. Invest., 1944, 23, 550.

Avidities and Titers of Blood Grouping Sera Prepared by Immunization of Donors with Group Specific Substances.

			Avidity	(seconds)		Agglutina	ation tite	 r
		Group "	B''cells	Group "	A" eellsf	Group '	'B'' cells	Group "	'A'' cells
Serum preparation		*	2*	*	**	*	**	*	**
Group "A" Group "B" Group "O"	No. 47 79 114 Pooled No. 73 19 98 Pooled No. 5 41 46 Pooled	20/65 35/120 27/75 20/50 27/75 27/75 	3/10 15/45 5/17 4/8 5/15 3/10 5/20 3/5	15/30 20/40 12/20 12/40 — 10/30 5/15 10/30	10/25 10/20 5/12 5/9 5/15 5/15 2/3 3/5	1:8 1:2 1:16 1:16 1:16	1:256 1:32 1:128 1:256 1:512 1:1024 1:256 1:1024	1:32 1:32 1:32 1:16 1:32 1:16 1:16	

^{*} Before immunization.

specific substances (0.2 mg A + 0.2 mg B). Three weeks after the injection the individuals were bled (500 cc) and their sera (stored under aseptic conditions in the refrigerator) were examined for titer and avidity. These were compared at the same time with control samples of sera (10 cc bleedings) obtained 1 week prior to the injection of group specific substances. The titers were determined by the serum dilution technic with 1% suspensions of washed (once) red cells. The agglutination response of the serum dilutions was observed after a 15-minute period of incubation at room temperature, followed by centrifugation for 1 minute at 2000 r.p.m. Readings were made macroscopically, and the endpoint reported as the greatest dilution giving a 4+ reaction, i.e., complete agglutination. Avidity determinations* were obtained by mixing equal volumes of a 10% suspension of once-washed red cells and serum on a slide. which was placed on a slide-agglutination viewing box.4 The viewing box was rocked slowly while observations were made macroscopically for the time required for the earliest

visible agglutination and then for complete agglutination to develop.

The results that are summarized in Table I show the avidities and titers of the individual and the pooled lots of sera. All of the individual bleedings (numbered sera) assayed at one time against the same red cell suspensions; the pooled sera of each group were assayed approximately one week later with other suspensions of red cells. There was a definite increase in the avidity and titer of the sera obtained from the immunized donors. The greatest increases in titer were observed in those individuals belonging to groups A and O. In the latter group sera were obtained that gave a 4+ reaction at a dilution of 1:1024 for both the anti-A and anti-B agglutinins. If the agglutination end point was taken as the greatest dilution showing some signs of agglutination, the titers of the more active sera would have values in the neighborhood of 1:4096. On the basis of a 4+ agglutination endpoint the titers obtained in our sera are at least as high as those obtained by Witebsky et al.1 However, due to the variability of the quantitative agglutination test among different laboratories, such a comparison cannot be made with any certainty. The greatest avidity response was obtained with

^{**} After immunization.

f Sub-group not determined.

^{*} We are indebted to Dr. L. K. Diamond of Harvard University for the technic of this determination and for the slide-agglutination viewing box that was employed.

⁴ Diamond, L. K., J. Lab. Clin. Med., 1945, 30, 204.

⁵ DeGowin, E. L., J. Clin. Invest., 1944, 23, 554.

he anti-B agglutinins of group A and O indiriduals. The pooled serat in almost every case were found to have titers and avidities

t We are very grateful to Dr. Wm. C. Boyd of Harvard University for comparing our pooled sera with the Harvard reference standards⁵ obtained as byproducts of plasma fractionation. The titers and avidities obtained by Dr. Boyd compare favorably with the reference standards. We also wish to thank Dr. Ernest Witebsky for kindly confirming the results of our assays.

approximating those of the strongest sera in the group. However, this point cannot be stressed much since the pooled sera were assayed against a different batch of red cells.

Conclusions. The increase in agglutination titer following immunization confirms the work of Witebsky¹ and Wiener.² In addition it has been shown that immunization with group specific substances increases the avidity, and that this increase roughly parallels the increase in agglutinin titer.

15172

Relationship of Turbidity to Acid Production by Lactobacillus arabinosus.

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In microbiological assays using the Lactobacillus arabinosus either turbidity or total acid production may be used as a measure of the response to the organism to various growth factors. Since, in all tests for which the organism is used, the turbidity resulting from bacterial growth increases with the amount of acid produced, it has generally been assumed that the turbidity corresponding to a given amount of acid production would be the same regardless of the growth factor to which the organisms were responding. In the course of some determinations of nicotinic acid, pantothenic acid, and biotin it was noted that the turbidity was much greater in relation to acid production when the organisms were responding to suboptimal amounts of nicotinic acid than when responding to suboptimal amount of biotin or calcium pantothenate.

Methods. All tests were carried out in triplicate except those involving *l*-tryptophane[†] which were done in duplicate. The organisms were grown in Evelyn photometer tubes in 10 cc of the medium of Snell and

Wright1 as modified by Krehl, Strong, and Elvehjem,2 omitting nicotinic acid, biotin, pantothenate, or l-tryptophane as the case might Stock cultures were carried and organisms were prepared for use as inocula as described by Snell and Wright.1 Turbidity was measured in the Evelyn photoelectric colorimeter using filter 660. The total acid produced was titrated with 0.1 N sodium hydroxide. Lactic acid was determined by the method of Barker and Summerson.3 These determinations were carried out in the biotin, nicotinic acid, and pantothenate tests after 18, 42, and 66 hours incubation at 37°C. In the tryptophane tests only the 66-hour determination was made and lactic acid production was not measured. The response to nicotinic acid was studied over the range from 0.1 to 1.0 µg, biotin from 0.1 to 2.0 millimicrograms, calcium pantothenate from 0.02 to 0.5 μ g, and l-tryptophane from 2 to 10 µg per 10 ml of medium

^{*} Present address: U. S. Public Health Service Hospital, Lexington, Ky.

t We are indebted to Dr. J. G. Wooley for these data.

¹ Snell, E. E., and Wright, L. D., J. Biol. Chem., 1941, 139, 675.

² Krehl, W. A., Strong, F. M., and Elvehjem, C. A., Ind. Eng. Chem., Analyt. Ed., 1943, 15, 475.

³ Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, 138, 535.

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			Avidity	(seconds)	Agglutination titer					
		Group "	B''eells	Group "	A" cellst	Group "	B" eells	Group "	Y" cells	
Serum preparation		*	**	*	**	*	**	*	**	
Group "A"	No. 47	20/65	3/10			1:8	1:256			
citatil.	79	35/120	15/45			1:2	1:32			
	114	27/75	5/17			1:16	1:128			
	Pooled	20/50	4/8			1:16	1:256			
Group "B"	No. 73	-,,	-, -	15/30	10/25			1:32	1:64	
oreal.	19			20/40	10/20			1:32	1:64	
	98			12/20	5/12			1:32	1:128	
	Pooled			12/40	5/9			1:16	1:128	
Group "O"	No. 5	27/75	5/13		5/15	1:16	1:512	1:32	1:256	
	41		3/10	10/30	5/15	1:32	1:1024	1:16	1:1024	
	46	15/60	5/20	5/15	2/3	1:16	1:256	1:16	1:128	
	Pooled	15/40	3/5	10/30	3/5	1:16	1:1024	1:64	1:1024	

^{*} Refore immunization.

specific substances (0.2 mg A + 0.2 mg B). Three weeks after the injection the individuals were bled (500 cc) and their sera (stored under aseptic conditions in the refrigerator) were examined for titer and avidity. These were compared at the same time with control samples of sera (10 cc bleedings) obtained I week prior to the injection of group specific The titers were determined by substances. the serum dilution technic with 1% suspensions of washed (once) red cells. The agglutination response of the serum dilutions was observed after a 15-minute period of incubation at room temperature, followed by centrifugation for 1 minute at 2000 r.p.m. Readings were made macroscopically, and the endpoint reported as the greatest dilution giving a 4+ reaction, i.e., complete agglutination. Avidity determinations* were obtained by mixing equal volumes of a 10% suspension of once-washed red cells and serum on a slide, which was placed on a slide-agglutination viewing box.4 The viewing box was rocked slowly while observations were made macroscopically for the time required for the earliest

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Diamond, L. K., J. Lab. Clin. Med., 1945, 30,

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^{**} After immunization.

f Sub-group not determined.

^{*} We are indebted to Dr. L. K. Diamond of Harvard University for the technic of this determination and for the slide-agglutination viewing box that was employed.

⁵ DeGowin, E. L., J. Clin. Invest., 1944, 23, 554.

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Though the total fermentation of carbohydrate is reduced when biotin, pantothenate, or nicotinic acid are present in suboptimal amounts, the fact that the end product is always lactic acid suggests that the main pathways of carbohydrate metabolism are unchanged regardless of the growth factor to which the organism is responding.

Summary. 1. The turbidity produced by

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15173

Changes in Erythrocytes of Hamsters Following Castration, Splenectomy, and Subsequent Liver, Iron, and Testosterone Injections.

KATHRYN F. STEIN AND EDITH CARRIER. (Introduced by A. Elizabeth Adams.)

From the Zoology Department, Mount Holyoke College.

A variety of evidence indicates that the male sex hormone has a stimulating effect upon the red blood cell count in mammals. It is well known that men have a higher average count than women, and a similar sex difference has been found to occur in many other mammals including rats,1 rabbits,2 mice,3 and cats.4 That this is due, in part at least, to the effect of the male hormone has been demonstrated by the increased count following testosterone injections in hypogonadic men,5 in capons,6 and in castrate female and hypophysectomized rats,7 and by the reduction in number of red blood cells following castration in the hamster (Cricetus auratus) reported previously from this labora-

tory.⁸ Some progress has also been made toward an understanding of the mechanism by which the effect of the male hormone is produced. Vollmer and Gordon⁷ found increased reticulocytosis and hyperplasia of the bone marrow in hypophysectomized rats injected with testosterone propionate. The present study was undertaken to confirm and extend the results obtained following castration in the hamster⁸ and also to attempt to discover further details concerning the processes involved in the effects of castration upon the red blood cells.

Experimental. Nineteen male golden hamsters (Cricetus auratus) (Tables I, II) were employed in this experiment, four sets of littermates plus 2 animals from the same strain but of which the exact relationship to each other was unknown. All were of the fourth and fifth generations from an original inbred litter of four animals born in this laboratory in the spring of 1943. They were kept in a temperature of 70 to 75°F and had Purina Dog Chow and/or Pratt's Nurishmix constantly available in their cages, supplemented occasionally by milk and lettuce. They appeared

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TABLE I. Relationship of Turbidity to Acid Production by Lactobacillus arabinosus.

Nicotini	e aeid	Caleium pa	utothenate	Biotin <i>l-</i> Tryptop		ophane	
μg per 10 ml medium	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Ratio* acid to turbidity	Milli- mierograms per 10 ml medium	Ratio* acid to turbidity	μg per 10 ml medium	Ratio* acid to turbidity	
	11.95	0.02	81	0.1	51.8	2	45.8
	13.0	0.04	78	0.2	51.6	4	44.4
	14.2	0.1	50.8	0.6	38.3	6	42.4
0.6	16.1	0,3	24.1	0.8	36.3	8	40.0
1.0	16.9	0.5	22.0	2.0	27.2	10	39.4

Total titratable acidity

2-Log, galvanometer reading

All figures were obtained after 66 hours incubation and are the average of triplicate determinations except in the instance of tryptophane, where they were done in duplicate.

Results. The results obtained at various incubation times were similar and varied only in degree. Therefore, only the results obtained at 66 hours incubation are reported.

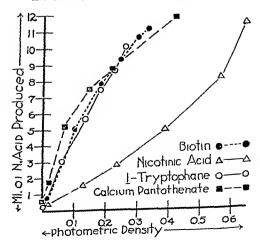
The ratios of total acid produced to photometric density were much lower when the organisms were responding to suboptimal amounts of nicotinic acid than when they were responding to suboptimal amounts of biotin, pantothenate, or l-tryptophane (Table I). At a level of acid production of 5 to 6 ml of 0.1 N acid (line 2 of Table I) the ratios were 13.0 for nicotinic acid, 78 for calcium pantothenate, 51.6 for biotin, and 44.4 for l-tryptophane. This indicates that, at this particular level of acid production, about 6 times as many bacteria were present in the nicotinic acid tubes as in the pantothenate tubes, and 31/2 to 4 times as many as in the tryptophane or biotin tubes. As the amount of nicotinic acid was increased the ratios rose, and as the amounts of pantothenate, biotin, and tryptophane were increased, the ratios fell and tended to approach those obtained with nicotinic acid. When photometric densities were plotted against total acid production (Fig. 1), the curves for biotin, pantothenate, and l-tryptophane overlapped and were quite similar, whereas the nicotinic acid curve differed from all the others.

Lactic acid accounted for 98 to 102% of the total titratable acid regardless of whether the organisms were responding to nicotinic acid, biotin, or pantothenate.

The data show that when Discussion.

biotin, pantothenate, or l-tryptophane are present in suboptimal amounts, fewer organisms are formed per unit of glucose metabolized than is the case with nicotinic acid. This suggests that lack of biotin or pantothenate interferes with the synthesis of protein to a greater degree than does lack of nicotinic acid. This view is reinforced by the similarity of the biotin and pantothenate curves to that of l-tryptophane. Conversely, a lack of nicotinic acid interferes with carbohydrate metabolism more than does a defi-

Figure -I-Relationship of Photometric Density to Acid Production by Lactobacillus Arabinosus



ciency of biotin, pantothenate, or l-tryptophane.

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TABLE I. Changes with Age in the Red Blood Cell Values of Hamsters.

		RBC ec	unt	\mathbf{R}	BC vol	•	Hemoglobin		
Age in days	Animal No.	No. of animals	Avg No. RBC and range in millions	No. of	Total vol. in %	MCV in μ ³		Total Hb. g 100 cc	MCHb in 77
20	1, 2	2	5.06 (5.03-5,08)						
33-34	3.7	จึ	5.96 (5.36-6,69)	1	47.5	70.8			
36-37	8, 12, 13, 14	4	6.36 (5.87-6.55)				3	17.04	27.1
40-43	3, 7, 14	3	6.98 (6.40-7.55)	2	45.3	67.5	1	17.8	23.6
47-50	3, 7, 14	3	7.94 (7.84-8.01)	2	43.8	55.2	1	18.3	22.9
57-62	3, 7, 14-17	6	8.60 (8.16-9,29)	6	52.9	63.2	4	17.38	20.9
66-70	3, 7, 9-11	5	8.88 (8.32-9.03)	4	48.6	55.1	7	18.94	20.4
85-100	3, 7, 11, 14, 15	5	9.14 (9.06-9.02)	5	52.2	57.1	4	18.26	19.9
108-131	3, 7, 11, 14, 15	5	9.17 (9.14-9.20)	5	53.2	58.0	2	15,65	17.1

to be in healthy condition throughout the experiments.

Castration was performed on 8 animals; 1 was operated at 20 days of age, 4 at 33 to 37 days, and 3 at 66 to 73 days (Table II). Controls were subjected to similar operative procedure up to the point of actual removal of the testes. Later in the experiment 4 of the castrates, all adult and with approximately the same number of red blood cells, and 5 controls, also adult, were splenectomized (Table II). Since no significant change had occurred due to operative procedure in the controls for the castrate animals, and since the removal of the spleen was accomplished with no greater loss of blood from hemorrhage, controls for the latter series were not operated. For all the operations 0.1 cc of a 10% alcoholic solution of Nembutal (Abbott), containing 1 gram per cc, was injected intraperitoneally as a preliminary anesthetic and followed by ether as needed.

Attempts to restore the counts of operated animals to normal were made by injections of liver extract or of iron citrate. The injected animals in each of the 2 groups, liver and iron, included 2 castrates, 1 splenectomized "control," 1 splenectomized castrate, and the same normal control (Table III). The liver (from Lederle Laboratories and containing 15 U.S.P. injectable units per cc) was administered in daily intramuscular injections of 0.1 cc or 1½ units to each animal for 3 days. Counts were taken within 12 hours after the last injection. Eli Lilly iron citrate, containing 0.05 grams per cc, was injected in 0.1 cc quantities into each animal of the "iron" group

daily for 3 days and counts taken within 6 hours after the last injection. Finally a series of 9 hamsters, 2 castrates, 3 splenectomized castrates, and 4 splenectomized "controls," were injected with 0.1 cc of a preparation containing one mg of propionic ester of testosterone per cc (Oreton Schering), daily for 3 days and counts taken the day following that of the last injection (Table IV).

Blood for all determinations was secured from the tails of animals under light ether anesthesia. An interval of a week was usually allowed between determinations to avoid the possibility of anemia from excess bleeding. A modified Hayem's solution recommended for rats by Smith9 was used as the diluent. Counts were made of 4 chambers and averaged for each reported count prior to the splenectomy operations; since the coefficient of variation was usually under 1% only 2 chambers were counted thereafter. Volume determinations were made in a Van Allen capillary hematocrit centrifuged at 2600 r.p.m., and hemoglobins were read in a colorimeter equipped with a Newcomer disc.

Results. Normal Levels of Young and Adult Hamsters. The number of red blood cells in the hamster increases with age (Table I). The data confirm the results previously reported on a smaller number of animals. The adult value of approximately 9 million per cc was a strikingly uniform result in all animals counted, and since it was also maintained in weekly counts up to the age of

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TABLE II.

Effects of Castration and Splenectomy on the Erythrocyte Count.

	Age at	operation]	Number of RBC in	millions
Animal No.	Castration	Splenectomy	Before op.	After castration	After splenectomy
1	20 days	119 days	5.08	6.41	6.52
$\overline{4}$	33		5.37	6.54	
8	37		6.52	6.57	
12	36	114	6.55	6.58	6.54
13	36	114	5.87	6.57	6.54
16	66	114	8.85	6.55	6.55
17	66		8.64	6.54	
9	73		9.00	6.56	
			A	vg 6.55	6.54
3		119	9.20		5.73
11		137	9.19		6.52
14		114	9.15		6.54
18		mature			6.52
19		"			6.56
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165 days (Stein and Jacobsen, 226 days) it is considered as the normal level for adult hamsters of this species. Increases in total red blood cell volume also occurred with age but not proportional to the increase in number of cells; consequently the size of the individual cells was perceptibly decreased. Variations in total hemoglobin were neither great nor consistent and mean corpuscular values were higher in young animals (Table I).

Effects of Castration and Splenectomy. A level of approximately 6.5 million cells per cu mm, as compared with the normal count of 9 million, was eventually attained by all castrated animals regardless of the age at which the operation was performed (Table II).

In adult animals this level was attained within a two-week period following castration and values within a range of 6.41 to 6.59 million were maintained for the 8-week period of counting. Counts for animals castrated before maturity rose to the 6.5 level and were then maintained for 1 to 6 weeks, that is, until other treatments were initiated or counting discontinued.

Removal of the spleen was followed by a marked reaction in normal control animals as compared with the lack of effect on castrates. The count for the former had dropped to an average of 6.4 million 2 weeks after the operation and this level was maintained for 6 weeks by the one animal for which weekly counts were made over so long a period. Also a

TABLE III.

Effects of Liver and Iron Injections on Red Blood Cells of Hamsters.

Animal			No. of RBC	in millions	Size of F	RBC in μ^3
No.	Operation	Injection	After op.	After inj.	After op.	After inj.
17	Castration	liver	6.54	9.06		
8	"	"	6.55	9.14	80.9	54.7
11	Splenectomy	,,	6.52	9.13	76.7	58.1
16	Castra. + splen.	"	6.54	9.19	76.3	53.3
15	Control	"	9.14	9.15	54.7	60.1
4	Castration	iron	6.54	8.85	85.6	
9	,,	21	6.56	9.11	81	
18	Splenectomy	"	6.52	9.17	75.2	54.5
13	Castra. + splenec.	"	6.57	9.07	84.5	59.3
15	Control	***	9.14	9.21	54.7	57.5

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$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	Again					RBC ro	1.	H	emoglob	in
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⁹ Smith, Christianna, J. Path. and Bact., 1932, 35, 717.

demonstrating a definite effect of the male sex hormone upon the number of red blood cells present in the circulating blood. As reported previously^S castration caused approximately a 25% decrease, from 9 to 6.5 million per cu mm, in the number of cells. This is similar to the decrease from 3.6 to 2.51 million observed by Taber, Davis, and Domm⁶ in capons. Injections of testosterone raised the number in castrated hamsters, as it did in capons⁶ and in hypophysectomized rats.^{7,10} In the latter the effect of the male hormone was noted as due to increased erythropoiesis. Because of the small amount of material available this has not been confirmed for the hamster.

Young animals responded somewhat differently from adults in that such animals, if they were castrated before the number of red blood cells had reached the adult castration level, showed an increase to that level. The final result was the same, a castration level of approximately 6.5 million cells per cu mm. The regulation of the gradual increase of the red cell count in young animals up to this level is apparently controlled by other than sex factors.

It is interesting that the change in the number of cells due to the influence of sex hormone was accompanied by a change in the size of the individual cells so that the total volume of red blood cells in the circulating blood was essentially the same in castrated as in normal animals. As far as our investigations go they seem to indicate that the total amount of hemoglobin was also kept at a relatively normal level. The increase in the amount of hemoglobin per cell in the castrates compensated on the whole for the decreased number of cells. The regulatory effect on the cell size and content thus seemed to be, in the hamster, the main effect of the male sex hormone.

The influence of the spleen on the blood picture has been the subject of numerous investigations. Both splenectomy and injections of splenic extract have indicated that the spleen may exert a stimulating effect upon the formation of red blood cells. 11.12,13 Splenectomy of hamsters in the present study produced the same decrease in the total number and the same increase in the size of individual

cells as was observed following castration. This similarity and the fact that splenectomy of castrated animals caused no further changes in the red blood cells suggested a possible interrelationship between the testis and the spleen. Evidence for such a relationship was furnished by the experiments in which injections of male hormone were made. No effect of restosterone on the number or size of red blood cells was observed in splenectomized animals, whether or not they had been castrated. The effect of male hormone in changing the count was apparently not exerted directly upon the bone marrow but upon the spleen. It is possible perhaps that the increase in number following testosterone injections was due, not to an indirect effect through the spleen on erythropoiesis, but merely to a direct stimulation of the spleen to release hoarded cells. Injections over longer periods than the 3 days of the present study might give different results. However, the positive results obtained with the use of liver or iron extract aiter a similar short period of injection at least indicate that the bone marrow is capable of response within that period.

It is further indicated from the results of injections of liver and iron that the effect of the spleen on the number and size of the red blood cells is tied up with the liver and/or iron supply since either liver extract or iron ' citrate was effective, within a period of 3 to 4 days, in restoring a normal count and a normal cell size in all operated animals, castrated. splenectomized, and those which were both splenectomized and castrated. It is difficult to understand why both substances should have the same effect. Liver deficiency anemias give a macrocytic picture as compared with the small size of the cells which ordinarily occur following iron deficiency.14 As noted previously, however, injections of iron citrate

¹⁰ Vollmer, Erwin P., Gordon, Albert S., and Charipper, Harry A., Endocrinol., 1942, 31, 619.

¹¹ Krumbhaar, E. B., and Musser, J. H., J. Exp. Med., 1914, 20, 108.

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^{105, 1176.}

		No. of RBC		Size of R	
Inimal No.	Operation	<i>Before</i> testos	After terone	Before testos	After terone
8	Castration	7.52	9.18		49.0
17	1)	7.73	9.12	****	
3	Splencetomy	8.09	7.60	64.1	
11	11	7.63	7.62		61.7
14	"	7.96	7.56	61.7	64.5
19	7.2	6.54	6.52		65,9
1	Castr. + splen.	8.25	7.34	63.0	62.7
$1\overline{2}$	3) "	7.01	7.62	65.6	64.3
16	,,	7.64	7.66		63.9

TABLE IV.
Effects of Testosterone Injections on Red Blood Cells of Hamsters.

count of 6.49 was made on the blood of another animal dying 10 months after splenectomy.

The average size of the erythrocytes increased both after castration and after splenectomy, in the former case to 81.2 (74.5-84.1) cubic micra, in the latter to 75.4 (70.3-80.3). Normal controls had an average cell size of 56.5 (51.4-60.9) cubic micra. Splenectomy of castrates was followed by a slight decrease, to 76 cubic micra, but it is doubtful if this difference is significant. In general the effects of splenectomy were essentially the same as those of castration.

Total hemoglobin values averaged slightly higher in castrate than in control animals but the difference was too slight to be significant. The amount of hemoglobin per individual cell, however, was definitely increased, ranging from 27 to 31 micromicrograms in castrates and from 17 to 21 in controls. The effect of splenectomy upon the total amount of hemoglobin in the blood of the hamster cannot be determined at this time. Data on individual animals, "normal" and castrate, before and after splenectomy, indicate a definite drop in amount of hemoglobin per 100 cc of blood both at one and two weeks after operation. However, unoperated controls, normal and castrate, showed a similar decrease when determinations were made on the same dates as those for the splenectomized animals. This may perhaps indicate a seasonal variation in hemoglobin.

Effects of Injections. Liver or iron citrate injected into the hamsters with lower than normal red blood cell counts resulted in an immediate rise of the number to normal,

whether the animals had previously been castrated, splenectomized, or both (Table III). These increases were noted within 6 to 12 hours after the last of 3 daily injections of the liver or iron. Along with the increased count a decrease occurred in the size of the individual cells (Table III).

Testosterone injected for a similar period, 3 daily injections, produced changes only in the castrated hamsters. None of the splenectomized animals, whether or not they had been castrated, showed any response in counts made the day following the last injection. The animals which received these injections had been treated previously with other substances and there had not been enough time for their counts to return to the level characteristic for their condition when the testosterone was injected. However, the effect, or lack of effect, of testosterone was obvious and consistent (Table IV).

In most of the splenectomized animals the number of red blood cells continued to drop toward the splenectomy level in spite of the testosterone injections and in none of them was there an increase in the least approaching the spectacular rise manifested by both of the castrates. The individual red blood cells remained macrocytic in all the splenectomized animals, castrated and uncastrated, injected with testosterone. In castrated "controls," however, the injections were followed by a decrease in average cell size in the one animal of this type (No. 8, Table IV) for which a volume determination was made.

Discussion. The results obtained on hamsters in this study agree with those of other workers in the field upon other animals in demonstrating a definite effect of the male sex hormone upon the number of red blood cells present in the circulating blood. As reported previously⁸ castration caused approximately a 25% decrease, from 9 to 6.5 million per cu mm, in the number of cells. This is similar to the decrease from 3.6 to 2.51 million observed by Taber, Davis, and Domm⁶ in capons. Injections of testosterone raised the number in castrated hamsters, as it did in capons⁶ and in hypophysectomized rats.^{7,10} In the latter the effect of the male hormone was noted as due to increased erythropoiesis. Because of the small amount of material available this has not been confirmed for the hamster.

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No. of RBC in millions Size of RBC in a3 Before Before After Animal No. Operation testosterone testosterone 49.0 8 Castration 7.52 9.18 7.73 9.12 17 ---64.1 3 Splenectomy 8,09 7.60 7.62 61.7 11 7.63 ., 7.56 61.7 64.5 7.96 14 3 7 65.9 6,54 6.52 19 62.7 63.0 Castr. + splen. 8.25 7.34 1 12 7.01 7.62 65.6 64.3 2 2 7.66 63.9 16 7.64

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as well as those of liver caused return of cell size and number to normal in the operated hamsters. It is true, of course, that the picture produced in the hamster following either or both operations may not be that of a true anemia, since the total volume of cells remains practically normal. Whether this is also true of the total hemoglobin cannot yet be stated definitely due to the complication of the results either by a seasonal variation of the pigment or by variation of technic. Krumbhaar¹⁵ however makes no distinction in kind. but points out that there are variations in degree and in time of appearance and duration of anemia following splenectomy of different animals. He suggests that the anemia is due to a loss with the spleen of a property which aids erythrocyte formation, such as the conservation of the iron of broken down erythrocytes. And Balliere16 noted that following splenectomy in the dog the iron content of the plasma dropped with a corresponding decrease in number of red blood cells. Presumably then the iron citrate injections may substitute for the absence of the spleen and may directly affect bone marrow. It is questionable whether the similar action of liver extract in this experiment can be attributed to its iron content since it is known that liver extract is not effective in remedying human anemia due to iron deficiency. However, perhaps the difference is a quantitative The only alternative explanation, if the same mechanism is supposed to be set in

motion by the two types of injections, is that the injected iron has an effect on the liver of the hamster resulting in the liberation of some liver substance more directly responsible for the effects on the bone marrow. Krumbhaar¹⁷ has also suggested that the spleen may have the mission of activating liver function.

Gordon et al. 18 questioned whether a function of the spleen might be to regulate the structure of the red cells as they are produced from the marrow. Certainly this study seems to indicate an influence of the spleen, and perhaps indirectly of the male hormone, on the regulation of the volume of the individual red blood cell.

Summary. Castration of hamsters caused a decrease in the number (25-30%) and in the size of erythrocytes. In animals castrated when young enough to have fewer cells than the number typical for castrates the count increased up to the castrate level. Splenectomy had the same effects as castration upon the number and size of red blood cells and no additional change occurred when castrated animals were splenectomized. Liver extract or iron citrate injected daily for three days restored a normal count and normal average corpuscular volume in all operated animals, castrates, splenectomized, and splenectomized castrates. Testosterone injected for a similar length of time was effective in castrates only and was ineffective in all splenectomized animals.

¹⁵ Krumbhaar, E. B., Am. J. Med. Sci., 1932,

¹⁶ Balliere, A., Biol. Abs., 1941, 152, 1640.

¹⁷ Krumbhaar, E. B., Physiol. Rev., 1926, 6, 160.
18 Gordon, Albert S., Kleinberg, William, and Ponder, Eric, Am. J. Physiol., 1937, 120, 150.

15174 P

Sparing Action of Protein on the Pantothenic Acid Requirement of the Rat.*

MARJORIE M. NELSON AND HERBERT M. EVANS.

From the Institute of Experimental Biology, University of California, Berkeley, Calif.

Studies on the requirement of the rat for pantothenic acid have, so far, been limited to diets containing a high proportion of carbohydrate with minimal levels of protein and fat. On these high-carbohydrate diets, it is well known that pantothenic acid deficient rats usually survive only 1 to 3 months. 1,2,3 (Table I.) The importance of the composition of the diet in relation to vitamin requirements is also well-known; e.g., less thiamine is required for animals maintained on high fat,3,3 and high protein 0,7,8 diets than for diets high in carbohydrate whereas more riboflavin is required for rats receiving diets high in fat,3,10

In the course of experiments with diets vary-

*Aided by grants from the Board of Research and from the Department of Agriculture of the University of California, and the Rockefeller Foundation, New York City. The following materials were generously contributed: crystalline B vitamins from Hoffmann-La Roche Company, Nutley, New Jersey, Lederle Laboratories, Inc., Pearl River, New York, and Merck and Company. Inc., Rahway, New Jersey; alpha-tacopherol from Merck and Company, Inc., Rahway, New Jersey.

t General Mills Fellow.

1 Mills, R. C., Shaw, J. H., Elvehjem, C. A., and Philhps, P. H., Proc. Soc. Exp. Biol. and Med., 1940, 45, 482.

² Salmon, W. D., and Engel, R. W., Proc. Soc. Exp. Biol. And Med., 1940, 43, 621.

3 Unna, K., J. Nutrition, 1940, 20, 565.

- 4 Funk, C., Z. f. Physiol. Chem., 1914, 89, 378. 5 Evans, H. M., and Lepkovsky, S., J. Biol. Chem., 1929, 83, 269.
 - 6 Banerjii, G. G., Biochem. J., 1941, 35, 1354.
- ⁷ Banerjii, G. G., and Yudkin, J., Brochem. J., 1942, 36, 530.
- ⁸ Wainio, W. W., Fed. Proc., 1942, 1, 87; J. Nutration, 1942, 24, 317.
- ⁹ Evans, H. M., Lepkovsky, S., and Murphy, E., J. Biol. Chem., 1934, 107, 443.
- 10 Mannering, G. J., Lipton, M. A., and Elvehjem, C. A., PROC. Soc. EXP. BIOL. AND MED., 1941, 46, 100.

ing in their proportions of carbohydrate, protein, and fat a marked sparing action of protein on the pantothenic acid requirement of the rat was discovered. When rats received diets deficient in pantothenic acid but containing 64% protein (casein) instead of 24% protein, growth was markedly accelerated and survival was greatly increased. This effect was observed both when the deficiency was instituted at birth and when it was instituted at weaning.

Experimental and Results. The basal (high carbohydrate) diet was composed of 64% sucrose, 24% alcohol-extracted casein, 8% hydrogenated cottonseed oil (Crisco), and 4% salts.11 The high protein diet contained 64% casein and 24% sucrose with the remainder of the constituents identical. Both diets contained crystalline B vitamins per kg diet: 5 mg thiamine HCl, 5 mg pyridoxine HCl, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg nicotinic acid, 400 mg inositol, and 1 g choline chloride. Each rat received weekly a fat-soluble vitamin mixture containing 325 mg corn oil (Mazola), 400 U.S.P. units vitamin A, 58 chick units vitamin D, and 3 mg alpha-tocopherol.

In the first series of animals, equivalent groups of male and female rats (Long-Evans strain) were placed on the deficient diets at weaning. Table I shows the marked effect of the high casein diet on growth and survival.

In the second series, litters of 6 young together with the mother were placed on the deficient diets the day of birth. The litters were weaned on the 21st day and continued on the same diet. This procedure results in a very acute deficiency¹² as can be seen in Table II. However, the sparing effect of the

¹¹ Salts No. 4 of Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem., 1941, 138, 459.

¹² Nelson, M. M., and Evans, H. M., in preparation.

Effect of Dictary Protein Level on Growth and Survival in Pautothenic Acid Deficiency Instituted at Weaning.

				Weaning.	m rancot	nenie Acid	Deficiency I	Instituted at
Exp. group % protein	No. of rats	Avg wt Day 21 g	Avg wt Day 60	Survival Day 60	Avg wt Day 90	Survival Day 90		Survival Day 120
24 64	13	47 48 47	84 110 100	54 80 58	93 110 120	8 50 42	g 94 132	0 10 25
	10 & 12 &	47 47	141 124	83 90 92	170 234 205	83 90 83	202 300 255	83 90 83

Effect of Dietary Protein Level on Growth and Survival in Pantothenic Acid Deficiency Instituted at

						Birth.	at in Pan	tothenic	Acid Def	iciency l	nstituted at
Exp. group % protein		of its	Arg wt Day 1	Avg wt Day 21	Survival	1	Survival Day 30	Avg wt		Avg wt	Surrival
34	40	ð	6.3	30			70	g	%	g	%
	38	ð	6.1	27	81	32 31	35	38	7	51	1
64	30	ŝ	6.0	31		0.7		32		•	
	30	ð	5. 9	30	88	31 30	76	53	37	83	14
										73	

64% casein diet on growth and survival is still clearly shown.

The superiority of the high protein diet may actually be due to the decreased level of dietary carbohydrate inasmuch as there is evidence that pantothenic acid is involved in carbohydrate metabolism.13 On the other hand, it may also be due to some component(s) of the casein such as (a) traces of pantothenic acid; (b) the amino acid content; (c) the phosphorus content; (d) unknown factors present which may be related to the utilization of pantothenic acid or to biotin and folic acid which affect pantothenic acid utilization.14,15 Extensive analyses of various purified and "vitamin-free" caseins have shown the presence of pantothenic acid in all samples, varying from 0.8 to 3.0 μg per gram.

However, experiments now in progress indicate that the pantothenic acid content of the casein in use is responsible for only part of the sparing action.

Summary. Rats maintained either from birth or from weaning on a pantothenic acid-deficient diet containing 64% casein grow more and survive longer than littermates maintained on a 24% casein-deficient diet under the same conditions. Several possible explanations for this sparing action are given.

¹³ Williams, R. J., Advances in Ensymology, 1943, 3, 253.

¹⁴ Wright, L. D., and Welch, A. D., Science, 1943, 97, 426.

¹⁵ Wright, L. D., and Welch, A. D., J. Nutrition, 1944, 27, 55.

15175

Effect of Atabrine on Tetrahymena geleii (Protozoa, Ciliata).

VINCENT GROUPÉ. (Introduced by Geoffrey Rake.)

From the Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N.J.

The development during recent years of pure, bacteria-free strains of protozoa has led to more precisely controlled physiological and nutritional studies. However, the effect of chemotherapeutic drugs on such pure cultures has been little studied. It seemed of interest, therefore, to study the effect of a synthetic antimalarial drug such as atabrine on the ciliate, *Tetrahymena geleii*, whose biochemical characteristics are known.¹

Methods. Bacteria-free cultures of Tetra-hymena geleii were obtained through the courtesy of Professor George W. Kidder. All cultures were maintained with strict asepsis on 1.5% proteose peptone at 25°C. Strain H¹ was selected from the 6 strains tested because it was slightly less resistant to atabrine.

Population counts were made using a Howard mold counting chamber on 1.0 ml samples of culture fixed with an equal volume of formalin. Five or more fields were counted per sample using that magnification and dilution resulting in 10 to 50 cells per field. Counts were recorded as the number of intact organisms per ml.

The effect of atabrine on the division rate was determined by adding 3.0 ml of a 24-hour culture of known population diluted 1-10 in 1.5% proteose peptone to duplicate tubes containing an equal volume of a serially diluted aqueous atabrine solution. The number of generations in 24 hours was calculated from the known population at 0 hours and the average population of the duplicate tubes after 24 hours incubation at 25°C. Sterile precautions were observed throughout the entire test.

Serial dilution tests were done as follows: to 0.5 cc of serial 2-fold dilutions of atabrine an equal volume of a 24-hour culture was added. After overnight incubation at room temperature the tubes were examined both microscopically for motility and macroscopically for turbidity. Although it was most important to use bacteria-free cultures it was found possible to disregard sterile precautions in performing the serial dilution test described above without altering the results of the test.

The atabrine dihydrochloride* used in these studies was sterilized by filtration through a chemically clean UF grade sintered glass filter.

Experimental. The effect of atabrine on the division rate of Tetrahymena geleii was studied first to determine the concentration necessary to inhibit actively growing cells. It will be seen from the data presented in Table I that a concentration of 100 γ per ml was lethal for all cells and that multiplication was inhibited by 50 γ per ml while little or no inhibition was observed in the presence of 25 γ of atabrine per ml. In tubes containing 50 γ per ml only 25% of the population was motile. This would indicate that a difference in resistance to atabrine exists among the population. Similar results were obtained in another experiment.

Inasmuch as direct observation showed that loss of motility was followed rapidly by swelling and disintegration of the cell in the above experiments, a simple serial dilution test (see methods) was used to determine the effect of age of the culture on the resistance of the cells to atabrine. Population counts, pH, and relative resistance to atabrine were determined on samples taken daily from each of 3 flask cultures over a period of 4 days. Comparable results were obtained on all 3 cultures and the pertinent data from one such culture is presented in Table II. It will be seen that the concentration of atabrine required to immo-

¹ Kidder, G. W., and Dewey, V. C., Physiol. Zool., 1945, 18, 136.

^{*} The atabrine dihydrochloride used was obtained through the courtesy of Dr. Arthur P. Richardson, Head of the Division of Pharmacology of the Squibb Institute for Medical Research.

TABLE I.

Effect of Dietary Protein Level on Growth and Survival in Pantothenic Acid Deficiency Instituted at Weaning.

Exp. group % protein	No. of rats	Avg wt Day 21 g	Avg wt Day 60 g	Survival Day 60 %	Avg wt Day 90 g	Survival Day 90 %	Avg wt Day 120 g	Survival Day 120
24	13 Q 10 & 12 &	47 48 47	84 110 100	54 80 58	93 110 120	8 50 42	94 132	0 10 25
64	12 Q 10 & 12 &	48 47 47	115 141 124	83 90 92	170 234 205	83 90 83	202 300 255	83 90 83

TABLE II.

Effect of Dietary Protein Level on Growth and Survival in Pantothenic Acid Deficiency Instituted at
Birth.

					~~~~						
Exp. group % protein	No ra	. of	Avg wt Day 1 g	Avg wt Day 21 g	Survival Day 21	Avg wt Day 30 g	Survival Day 30	Avg wt Day 45	Survival Day 45 %	Avg wt Day 60 g	Survival Day 60 %
24	40	ô	6.3	30	81	32	95	38	7	51	1
	38	δ	6.1	27	91	31	35	32	,		•
64	30	ô	6.0	31	20	31	<b>#</b> 0	53	Α.	83	14
	30	₽	5.9	30	88	30	76	44	37	73	41

64% casein diet on growth and survival is still clearly shown.

The superiority of the high protein diet may actually be due to the decreased level of dietary carbohydrate inasmuch as there is evidence that pantothenic acid is involved in carbohydrate metabolism.13 On the other hand, it may also be due to some component(s) of the casein such as (a) traces of pantothenic acid; (b) the amino acid content; (c) the phosphorus content; (d) unknown factors present which may be related to the utilization of pantothenic acid or to biotin and folic acid which affect pantothenic acid util-Extensive analyses of various ization.14,15 purified and "vitamin-free" caseins have shown the presence of pantothenic acid in all samples, varying from 0.8 to 3.0 µg per gram.

However, experiments now in progress indicate that the pantothenic acid content of the casein in use is responsible for only part of the sparing action.

Summary. Rats maintained either from birth or from weaning on a pantothenic acid-deficient diet containing 64% casein grow more and survive longer than littermates maintained on a 24% casein-deficient diet under the same conditions. Several possible explanations for this sparing action are given.

¹³ Williams, R. J., Advances in Enzymology, 1943, 3, 253.

¹⁴ Wright, L. D., and Welch, A. D., Science, 1943, 97, 426.

¹⁵ Wright, L. D., and Welch, A. D., J. Nutrition, 1944, 27, 55.

		TABI	LΕ	III.		
Effect of	to Hq	Culture	on	Resistance	to	Atabrine.

		Atabrine—7 per ml												
pH of culture	5,000	2,500	1,250	625	313	156	78	39	19.5	9.8	4.9	2.5	1.3	0
6.35 6.60 6.80 6.95 7.18 7.48	1+ 0 0 0 0	4+ 1+ 0 0 0 0	4+ 1+ 0 0 0 0	4+ 2+ 0 0 0 0	4+ 0 0 0 0	4+ 4+ 0 0 0 0	1+ 1+ 1+ 0 0	4+ 4+ 4+ ± 0	1+ 1+ 1+ 1+ 0	4+ 4+ 4+ 4+ 0	4+ 4+ 4+ 4+ 4+ 0	4+ 4+ 4+ 4+ 4+ 3+	1+ 1+ 1+ 1+ 1+ 1+	4+ 4+ 4+ 4+ 4+ 4+

TABLE IV. Effect of Conditioned Medium and Frozen Cells on Resistance to Atabrine.

			Atabriue—y per ml							
24-br culture plus an equal volume of:	$\mathbf{pH}$	500	250	125	63	31	16	8	0	
Fresh medium	7.0	0	0	0	1+	4+	4+	4+	4+	
Conditioned medium*	7.0	0	0	0	0	0	4+	4+	4+	
Frozen cell suspensiont (cellular debris)	7.0	0	0	0	0	0	2+	4+	4+	

^{*} Cell-free supernate from 6-day-old culture adjusted to pH 7.0.

sented in Table IV show that at pH 7.0 the resistance of a 24-hour-old culture was significantly decreased by the addition of an equal volume of conditioned medium (obtained by adjusting the cell-free supernate from a 6-day-old culture to pH 7.0) or a frozen cell suspension containing only cellular debris.

That the decreased resistance of cells from older cultures is the result of environmental changes is shown by the fact that when 24-hour- and 9-day-old cells were resuspended in fresh medium to contain 122,000 and 140,000 cells per ml respectively at pH 6.9 a concentration of 63  $\gamma$  of atabrine per ml immobilized approximately 50% of the population in each case.

Although, as shown in the experiments described above, the resistance of Tetrahymena geleii to atabrine was influenced by many factors it was possible to obtain quite reproducible results using 24-hour bacteria-free cultures in the logarithmic phase of growth at pH 6.9 to 7.0. A test similar to that used for the quantitative determination of antibiotic activity³ was used and performed

as follows. Two ml of culture was added to a series of Wassermann tubes each of which contained 20% less atabrine than the preceding tube. After overnight incubation at 25°C the endpoint was recorded as the highest dilution of atabrine in which no macroscoic turbidity was observed (although motile cells were present). Providing a bacteria-free culture was used the endpoint was not affected when sterile precautions were disregarded in performing the test. In a series of 14 replicate titrations performed over a period of one month, according to the technic described above, an endpoint of 40 to 43  $\gamma$  of atabrine was obtained in every case. Bacteria-free cultures of Tetrahymena geleii are particularly well suited for such studies because conjugation does not occur and endomixis has not been observed.4

Summary. Multiplication of Tetrahymena geleii was inhibited by 50  $\gamma$  of atabrine per ml. Resistance of the cells to atabrine was progressively decreased by the environmental changes occurring during growth of the culture and was strikingly decreased when the pH of the culture was increased.

^{† 24-}hour-old cells resuspended in 1/3 volume of fresh medium then frozen and thawed.

³ McKee, C. M., Rake, G., and Menzel, A. E. O., J. Immunology, 1944, 48, 259.

⁴ Kidder, G. W., personal communication.

		TABL			
Effect	of	Atabrine	on	Division	Rate

Atabrine,	Intact org:	ınisms per ml	Calculated No.	% of population motile after 24 hr	
gamma per ml	0 hr	24 hr	of generations in 24 hr		
400	4300	0			
200	"	ň		v	
100	"	ň*		U	
50	,,,	3,000	0.7	0	
25	2;		0.5	25	
12.5	,,	24,000	2.5	100	
6.3	"	24,400	2.5	100	
3.1	,,	24,000	2.5	100	
		31,200	2.8	100	
1.6	"	33,200	3.0	100	
0.8	"	39,000	3,2	100	
0	,,	37,000	3.1	100	

^{*} No growth on subculture.

TABLE II.

Effect of Age of Culture on Resistance to Atabrine

Age of culture.	Population, cells/ee					Atab	rine—y	per m	l		
Hr	× 1000	$p\Pi$	250	125	62.5	31.3	15.6	7.8	3.9	1.95	0
1	2	6.9	0	0	1+	4-4-	44				44
24	30.6	7.1	Ō	ŏ	õ'	14	1+				1-1
48	43.4	7.2	0	õ	i)	ō'	0 1	4-4-	4-4-		4-
72	127	7.6	0	Ò	Ŏ	Ď	Ü	+	1-1-	14-	44
96	114	7.7	Ó	0	Ō	Õ	õ	=	<u>.</u>	<u>-</u>	4-1

^{0 =} No motile cells observed.

bilize all cells progressively decreased from 125  $\gamma$  per ml 1 hour after the flask was inoculated to 15.6  $\gamma$  per ml 72 hours after inoculation. In order to determine what factors might be responsible for this marked decrease in resistance to atabrine the experiments described below were carried out.

Size of the population did not appreciably affect resistance to atabrine since a 32-fold dilution of washed cells from a 24-hour culture had little or no effect on the concentration of atabrine required to immobilize the cells. However, as shown in Table II, the pH increased from 6.9 to 7.6 during 72 hours of incubation. Since an increase in pH resulted in more effective inhibition of Escherichia coli by atabrine,² it was of interest to study the effect of the pH of the culture on the resistance of Tetrahymena geleii to atabrine.

It will be seen (Table III) that the resistance of a 24-hour culture to atabrine decreased at least 1,000-fold when the pH of the culture was increased from pH 6.35, where the cells survived a concentration of 5,000 y or more of atabrine per ml, to pH 7.48 where only 4.9 y per ml were required to immobilize all cells. In each of 2 additional experiments resistance to atabrine was strikingly decreased when the pH was increased although it was not possible to predict the lowest inhibitory concentration of atabrine from the pH of the culture. It was also found that when a 9-day-old culture at pH 7.8 was adjusted to pH 6.9 the resistance to atabrine only increased from 4 to 16  $\gamma$ per ml.

It was of interest to determine whether, aside from the increase in pH, the medium was altered during growth by the accumulation of metabolic products or cellular debris in such a way as to decrease the resistance of the cells. The results of a single experiment pre-

^{± =} Occasional motile cell.

^{1+ =} Approximately 25% of cells motile.

^{2+ = &#}x27;' 50% '' '' '' 3+ = '' 75% '' '' ''

^{4+ =} All cells motile.

² Silverman, M., and Evans, E. A., J. Biol. Chem., 1944, 154, 521.

TABLE I.
Therapeutic Effect of Streptomycin in Experimental Murine Pertussis.

		of surv group of org	of 4	mice		M.L.D. of millions of organisms	% of survivors with negative lung cultures	Ratio of difference in % to its S.E.
Exp. I (Schieven Strain) Streptomycin ² Saline (controls)	125 3 0	31.2 4 1	7.8 4 4	1.9 4 3	0.47 4 3	>125 12.7	73 18	3.4 to 1
Exp. II (Rizzo Strain) Streptomycin* Saline (controls)	0 <del>1</del>	4	4	4	7	>125 39	100 78	2 to 1

^{*} Lot 228-0.2 ml (130 units) injected subcutaneously 4 × daily for 41/2 days. Total dose for each mouse: 2340 units.

TABLE II.
Therapeutic Effect of Streptomycin and of Penicillin in Experimental Murine Pertussis.

			of 4	mice		M.L.D. of	% of survivors	Ratio of difference in % to
(Sharpe Strain)	125	31.2	7.8	1.9	0.47	organisms	with negative lung cultures	its S.E.
*Streptomycin †Penicillin	4 0	4 0	4 1	4	4 2	>125	100 29	4.4-1
Saline (controls)	0 7	<b>4</b> 0	4 1	1	4 3	>125 1.2	95 20	4.0-1

^{*} Lot 228—Each mouse received intraabdominally 0.25 ml (250 units) 4 × daily for 5 days. Total dose: 5,000 units per mouse.

of 11 untreated survivors had negative cultures. This difference in the percentages of negative cultures obtained from the 2 groups is statistically significant, for the ratio between the difference and its standard error is 3.4 to 1. In the second experiment (Table I) the clearance effect was similar, though not so definite as in the first experiment (ratio: 2 to 1).

The data of Table II concerns a third experiment in which the protective effect of streptomycin was compared with that of penicillin. It is clear that streptomycin was again definitely protective while penicillin

failed. When the 2 antibiotics were combined, the result was similar to that of streptomycin alone.

The protective action of streptomycin as indicated in the above experiments was sufficient to justify a trial in the human disease, studies of which are now in progress.

Summary. Streptomycin exerts a protective therapeutic effect on the course of experimental murine pertussis as indicated by a significant reduction in the mortality rate and the disappearance of the organisms from the lungs of the surviving mice.

[†] Lot 228-0.25 ml (250 units) injected subcutaneously  $4 \times$  daily for 5 days. Total dose for each mouse: 5000 units

[†] Lot 299—(Cheplin) Each mouse received intra abdominally 0.25 ml (5 units)  $4 \times$  daily for 5 days. Total dose: 100 units per mouse.

Each mouse received intraabdominally 5 units of penicillin and 250 units of streptomycin  $4 \times daily$ , for 5 days. Total dose: 100 units of penicillin + 5,000 units of streptomycin.

## Therapeutic Effect of Streptomycin in Experimental Murine Pertussis,*

### WILLIAM L. BRADFORD AND ELIZABETH DAY.

From the Department of Pediatrics, the University of Rochester School of Medicine and Dentistry.

Streptomycin, an antibiotic discovered by Waksman and his associates, is produced by the growth of certain strains of Actinomyces griseus in a suitable medium. Because it exhibits activity against certain gram-negative organisms and is relatively non-toxic, it seemed to us important to test its value in the treatment of experimental murine pertussis as a preliminary to its use in the human disease. The present report describes the therapeutic effect of streptomycin on the course of the experimental disease in mice.

Materials and Method. A standard type of experimental infection in mice,² used for several years in this laboratory, was employed. It consisted of the intranasal inoculation of groups of standard 3-week-old white Swiss mice with freshly isolated strains of Hemophilus pertussis.

The 48-hour growth of H. pertussis was scraped from the surface of Bordet medium into 0.85% salt solution, thoroughly mixed, strained through gauze and standardized to contain approximately 10 billion organisms per ml. Four-fold dilutions of this suspension were prepared for the inoculation of subgroups of mice. Under light ether anesthesia. each mouse, held in the upright position, received an intranasal instillation of 0.05 ml of the organism suspension. Usually 5 subgroups, each consisting of 4 mice, were inoculated. The dose of organisms given to the mice of the various sub-groups was approximately 125, 31.2, 7.8, 1.9, and 0.47 million respectively.

After inoculation the mice were kept under uniform environmental conditions. Deaths

* Supported in part by a grant from The John

were recorded daily for a 10-day period, at the end of which period the surviving mice were autopsied and cultures of the lungs made. The method of Reed and Muench³ was used to calculate the 50% endpoint, indicating the minimal lethal dose (MLD) for each group of 20 mice.

Streptomycin hydrochloride (Lot 228) was supplied for these experiments by Merck and Co. through the courtesy of Dr. D. F. Robertson at the request of Dr. S. A. Waksman who had kindly supplied us with material for preliminary experiments. It contained 350 units per milligram of dry material and was dissolved and diluted to appropriate concentration in sterile distilled water. Treatment was instituted 4 hours after the mice were infected.

Sodium penicillin (Lot 299) (Cheplin), in the crystalline form, contained 100,000 Oxford units per 60 mg and was prepared for injection in a similar manner.

Results. Under the conditions of the experiments it is clear that streptomycin was definitely protective against the experimental disease. As indicated by the data (Table I), it required more than 10 times the number of organisms (Schieven Strain) to kill 50% of the treated mice than the untreated. When another strain (Rizzo) was used, the protection ratio was more than 3 to 1 in favor of the treated group. In addition to the difference of the mortality rates between the treated and untreated groups there was a distinct difference between the percentages of surviving mice showing negative lung cultures.

In the first experiment (Table I) 14 (73%) of the 19 surviving treated mice had negative lung cultures, indicating a rapid clearance of the organisms from the lungs as a result of treatment with the antibiotic. Only 2 (18%)

and Mary R. Markle Foundation.

1 Schatz, A., Bugie, E., and Waksman, S. A.,
Proc. Soc. Exp. Biol. And Med., 1944, 55, 66.

² Bradford, W. L., Brooks, A. M., and Katsampes, C. P., Yale J. Biol. and Med., 1944, 16, 435.

³ Recd, L. J., and Muench, H., Am. J. Hyg., 1938, 27, 493.

To provide simpler experimental conditions, an electric current having parallel isopotential lines and appropriate current density must be passed up or down the polar axis of the root and must coincide with the orientation of the general axis of the inherent electric field of the root as well as that of gravity.

An apparatus was designed to comply with this requirement and to maintain the roots under normal conditions of growth except for the applied current. Three sets of roots were used in each experiment: (1) control roots, (2) roots through which an electric current was passed upward, and (3) roots in which the same current was passed down-Since individual roots vary slightly with respect to the threshold of current density for reversible inhibition of growth and also in their rates of elongation, each experiment involved 3 or more duplicate sets of roots. Preliminary experiments led to a choice of a current giving an IR drop of 40 mV/mm, with the root immersed in tap water as the growing medium and the current passed upward through 25 mm of the apical end of the growing root. This gives complete inhibition of growth. The same current passed downward gives a very slight inhibition of growth, Fig. 1, or none at all At 37.8 mV/mm, complete but reversible growth inhibition is obtained with ascending currents.

Most experiments were divided into 3 periods: normal growth rate, current passing, and current off. The growth of each root was recorded under the microscope throughout the experiment.

Regions of the Growing Root Affected by the Electric Current. To determine what region of the growing root apex is involved in the growth response and inhibited by the current, a set of roots was marked in the usual way by means of particles of India ink. The results of one experiment are drawn to scale in Fig. 1. Ordinates are mm length, abscissa hours. I, II, and III are triplicate sets. A current of 40 mV/mm was passed up roots A, down roots B, and the right-hand group were controls It is evident from the measurements that the growth process under these conditions was prevented by an ascending current in both the region of active cell division and the region of cell elongation.

The inherent E.M.F.'s generated by the root are so oriented that the ascending current, which causes inhibition, opposes them. An applied current (field) of like intensity, but oriented with, rather than against the inherent one, has little or no effect. A detailed report of all experiments will be presented elsewhere.

#### 15178 P

#### Effect of Induced Pain on Pain Threshold.*

C. M. Parsons and F. R. Goetzl. (Introduced by Harold S. Olcott.)

From the Department of Medical Research, Permanente Foundation Hospital, Oakland, Calif.

Relief of pain by counterirritation is common experience and frequently was utilized therapeutically by the older physicians. The number of experimental studies reported in the literature is too small to permit conclusions to be drawn regarding mechanisms involved in the phenomenon which thus remains but poorly understood. Therefore reinvestigations on a larger group of subjects appeared desirable.

Methods. Pain threshold determinations

were performed in a group of 14 normal human subjects, men and women, ranging in age between 19 and 45 years. The algesimetric method employed has been described in detail elsewhere.¹ It consisted of applying an

^{*} This work was aided in part by a grant from Whitehall Pharmacal Company.

¹ Goetzl, F. R., Burrell, D. Y., and Ivy, A. C., Quart. Bull. Northwestern Univ. Med. School, 1943, 17, 280

#### 15177 P

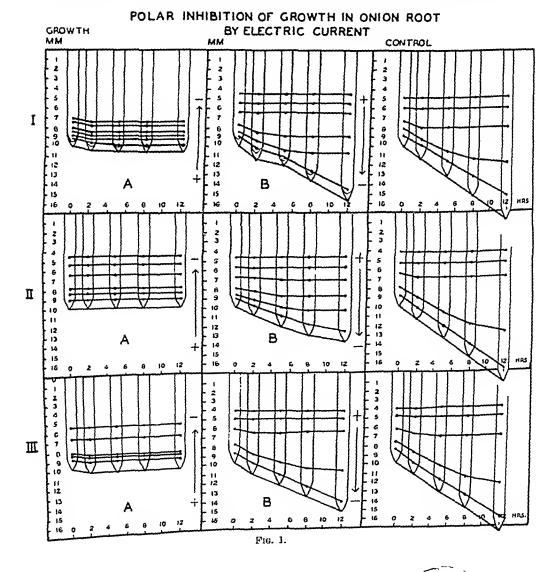
## Electric Control of Polar Growth in Roots of Allium cepa.

E. J. LUND, R. I. MAHAN, AND A. H. HANSZEN.

From the Laboratory of Biophysics, University of Texas.

Numerous experiments have been performed to determine the effects of continuous electric currents passed through growing roots immersed in various aqueous media. In practically all, the electric field was oriented at right angles to the long axis of the root. Under such conditions a minimum of two diverse stimuli, viz., gravity and applied E.M.F., operate at right angles to one another, thus causing a complicated type of variable growth response.^{1,2}

² Navez, A. E., J. Gen. Physiol., 1927, 10, 551.



¹ Elfving, F., Bot. Z., 1882, 40, 258.

To provide simpler experimental conditions, an electric current having parallel isopotential lines and appropriate current density must be passed up or down the polar axis of the root and must coincide with the orientation of the general axis of the inherent electric field of the root as well as that of gravity.

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were performed in a group of 14 normal human subjects, men and women, ranging in age between 19 and 45 years. The algesimetric method employed has been described in detail elsewhere.¹ It consisted of applying an

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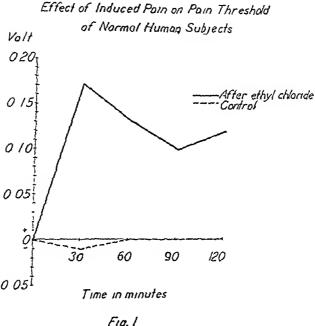


Fig. 1

alternating current to a tooth through a metal filling and noting the voltage at which the subject first experienced a painful sensation. Such voltage was considered an expression of the pain threshold level.

Pain was produced by spraying ethylchloride to an area of one square inch over the anterior surface of the subjects' tibiæ for a period of 20 seconds. At that time pain of burning or aching character was complained of in all instances. Pain thresholds were determined shortly before and at 30-minute intervals for 2 hours after the application of ethylchloride.

Results. Fig. 1 shows the averages of the values obtained in the determinations of pain threshold, expressed in terms of the mean changes in voltage required to produce a painful sensation in the subjects' teeth. The control values were obtained from 34 control tests in the 14 subjects and were found to be between 0.2 and 1.8 volt. Values after application of ethylchloride were obtained from 21 experiments in 11 of the 14 subjects. Analgesic effects were observed in all instances. The greatest variation in pain threshold during control periods in every subject was sig-

nificantly smaller than the smallest change of threshold following application of ethylchloride. Therefore the curves presented give an indication of the results obtained for each subject.

In the control determinations the mean change in threshold value for the entire group was minus 0.003 volt; the corresponding value for the period following application of ethylchloride was plus 0.129 volt.

Comment. The observations reported are in partial agreement with results obtained by other authors,2 They, however, employed different algesimetric procedures and different types of induced pain. Also, their studies were made on only 3 subjects and the results were expressed in percentages rather than in actual changes in threshold values. The pain threshold was reported to be elevated by induced pain by approximately 30%.

It is interesting, also, to compare the present results with those obtained previously in studies on analgesic effects of morphine sulfate in normal human subjects using the pres-

² Hardy, J. D., Wolff, H. G., and Goodell, H., J. Clin. Invest., 1940, 19, 649.

ent method.³ Not all of the subjects tested showed an elevation in pain threshold following administration of 0.016 g of morphine sulfate, which for the entire group produced a mean increase of only 0.0218 volt.

The fact that for producing analgesic effects in the present experiment ethylchloride had to be applied until pain developed indicated that pain rather than cold was the sensation responsible for elevating pain thresholds. The mechanisms involved in producing the analgesic effects cannot yet he stated with certainty. Distraction, changes in local blood supply and other short lasting effects of induced pain cannot fully explain the phenomenon because the pain induced in our suhjects lasted for 2 to 3 minutes at the most while the analgesic effects were noticeable for

a period of 90 to 120 minutes.

In additional investigations, the possibility is being examined whether epinephrine might be the agent involved in producing analgesic effects following induced pain. The trend of thought seems logical in view of the facts that epinephrine is released by pain from the adrenal glands⁴ and that it possesses marked analgesic properties when injected in man or dog.⁵

Summary. Experiments have been made in normal human subjects which demonstrate that induced pain of short duration produces long lasting analgesic effects.

#### 15179

# Effect of Adrenocorticotropic Hormone (ACTH) on Hypophysectomized Adrenal-demedullated Rats.*

MIRIAM E. SIMPSON, CHOH HAO LI, AND HERBERT M. EVANS.

From the Institute of Experimental Biology, University of California, Berkeley.

Ingle¹ has raised the question of the possible role of the adrenal medulla in the response of the adrenal cortex to adrenocorticotropic hormone. In the present study the problem of the relationship hetween the two parts of the adrenal has heen approached hy comparing the effectiveness of adrenocorticotropic hormone as a stimulant of the cortical tissue when administered in the presence or absence of the medullary tissue.

Methods. Male rats were adrenal-demedullated[†] at 25 days of age and the adrenal cortex was allowed to regenerate for 16 days. The animals were supported for the first 13 days of this period hy 1% NaCl in the drinking water. The rats were then hypophysectomized, at 41 days of age. Pure ACTH² was injected for 15 days, heginning on the day of hypophysectomy, in an effort to maintain the adrenal cortical tissue. The cortical response of the douhly operated animals was compared with that of an equal number of rats hypophysectomized at the same age, in which the adrenal was intact. The daily dose of ACTH, 0.2 mg, was divided into two intraperitoneal injections of 0.5 cc each.

At autopsy the sella turcica of all hypophysectomized rats was searched under 10-fold magnification for remnants of pituitary tissue.

³ Ivy, A. C., Goetzl, F. R., and Burrill, D. Y., War Medicine, 1944, 6, 67.

⁴ Cannon, W. B., Bodily Changes in Pain, Hunger, Fear, and Rage, New York, D. Appleton and Co., 1929.

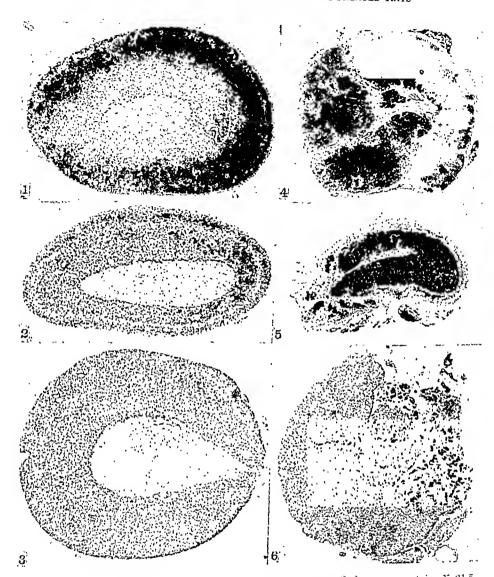
⁵ Ivy, A. C., Goetzl, F. R., Harris, S. C., and Burrill, D. Y., Quart. Bull. Northwestern Univ. Med. School, 1944, 18, 298.

^{*} Aided by grants from the Research Board of the University of California, the Rockefeller Foundation, and the Josiah Maey, Jr., Foundation.

¹ Ingle, D. J., Endocrinol., 1942, 31, 419.

t The adrenal medulla and all cortical tissue except a few cells adhering to the capsule were enucleated.

² Li, C. H., Evans, H. M., and Simpson, M. E., J. Biol. Chem., 1943, 149, 413.



Sudan orange stain, X 21.5. Fig. 1. Adrenal of a normal 41-day old male rat, illustrating the condition of the adrenal cortex at the time of hypophysectomy. Note the even distribution of fine lipid droplets, with a slightly greater concentration in the glomerulosa and a faint subglomerular zone of somewhat

depleted lipid content. Weight of 2 adrenals 25 mg.

Fig. 2. Adrenal of a 56-day-old hypophysectomized male rat, 15 days postoperative. The cortex is markedly reduced in width. The lipid occurs in coarse droplets and there is a wide

subglomerular sudanophobe zone. Weight of 2 adreuals 10 mg.

Fig. 3. Adrenal of a 56-day-old hypophysectomized rat treated with 0.2 mg of pure ACTH daily for 15 days, beginning on the day of hypophysectomy at 41 days of age. The cortex has been completely maintained and even slightly hypertrophied, as shown by the strikingly uniform distribution of lipid throughout its entire width, obliterating even the narrow subglomerular zone of the normal male. Weight of 2 adrenals 22 mg.

Fig. 4. Regenerated adrenal cortex of a 41-day-old male rat which was adrenal-demedullated at 25 days of age. The cortical tissue has reorganized in the normal zonal relationships around a central vein. The narrow subglomerular zone of lipid depletion characteristic of the normal

male is discernible. Weight of cortical tissue 12 mg. Fig. 5. Regenerated adrenal cortex of a 56-day-old male rat which was adrenal-demedullated



at 25 days of age and hypophysectomized at 41 days of age. During the 15 days chapsing since the second operation, the typical pattern of hypophysectomy has been superimposed upon the regenerated cortex. Weight of cortical tissue 8 mg.

Fig. 6. Regenerated adrenal cortex of a 56 day old male rat which was adicual demodullated at 25 days of age, hypophysectomized at 41 days of age and treated, beginning immediately after operation, with 02 mg of pure ACTH daily for 15 days. The contreal lipid distribution is eutirely comparable to that seen in the injected hypophysicomized animal with intact adicuals, and the same evidence of hypertrophy is seen in the obliteration of the subglomerular zone (Fig. 3). Reorganization of the regenerated cortical tissue has advanced as much under the influence of ACTH as in the presence of the pituitaly. Weight of contreal tissue 17 mg

Any questionable tissue was serially sectioned and examined Atrophy of the thyroid and gonads gave additional evidence for complete removal of the pituitary. The adrenal tissue of the "demedullated" rats was sectioned serially and examined for remnants of medullary Any animals in which fragments of pituitary or adrenal medullary tissue had been left at operation have been eliminated in reporting the results of the experiment.

A total of 42 rats was used. It was necessary to provide appropriate controls for each important period in the experiment, in order to establish: (1) the extent of cortical regeneration 16 days after adrenal demedullation, at which time hypophysectomy was performed and injections were begun; (2) the condition of the adrenal cortex of the normal animal at that time; (3) the effect of hypophysectomy upon both regenerated and normal cortical tissue, as seen 15 days postoperatively, at the end of the experiment; (4) the amount of additional cortical regeneration which had taken place in the demedullated animal possessing a pituitary, during this 15-day period Accumulated data on normal 56-day-old rats were available for the controls at the end of the experiment

The distribution of animals into experimental and control groups may be seen in

Table I. Initially there were 6 animals in each group.

Results. As will be seen in Table I, there was no difference in cortical weight response to ACTH conditioned by the presence or absence of medullary tissue. The dose used, 02 mg daily, was adequate to maintain the adrenal weight at the time of hypophysectomy in both types of experimental animal. thymus was also weighed as a further measure of cortical response, and its reduction was found to have been equally drastic in both types of animal receiving ACTH.

Frozen sections of the adrenals, stained with Sudan orange, are illustrated in Fig. 1 to 6. and the salient features of cortical lipid distribution are described there The regenerated cortical tissue in demedullated adrenals was protected against the effects of hypophysectomy by this dose of ACTH as completely as was the cortex of intact adrenals (Fig 3 and 6). Although the weights had not been increased above the operative level, the adrenal cortex in both hypophysectomized and doubly operated animals showed some evidences of hypertrophy Vividly sudanophilic lipid, in droplets slightly larger than normal, was distributed evenly throughout the entire width of the cortex, obliterating even the narrow subglomerular zone characteristic

TABLE I. Response of Hypophysectomized Rats to ACTH in the Presence of Absence of the Adrenal Medulla

		Adıeı	ıals ıntaet		1	di enals	demedullat	ed
Treatment	No of	Body wt g	Adrenals wt, mg		No of rats	Body wt, g	Cortical tissue, ing	Thyum:
Controls at time of hypophy sections 41 days old Hypophysectomized, ACTH	6	133	25	304	6	139	17	402
for 15 days  Hypophysectomized controls at autopsy, 15 days post	6	105	24	63	6	115	17	91
operative Controls at autopsy, 50 days old	6 54	115 215	10 34	196 451	$\frac{2}{6}$	124 215	10 22	199 436

of the normal male.‡ The cortical tissue of the ACTH-treated doubly operated animals seemed, in addition, to have undergone reorganization beyond that seen at the time of hypophysectomy; it resembled more closely the condition in the demedullated controls at autopsy, in which the cortical tissue had been continuing regeneration under the influence of the animal's own pituitary.

Conclusions. The stimulating action of ACTH on the adrenal cortex of hypophysec-

tomized rats, as far as could be judged by adrenal weights and by morphology as seen in lipid stains, was the same in the absence of the medulla as in the intact adrenal.

‡ This is the typical picture of adrenal hypertrophy which has been obtained upon administration of adequate doses of ACTH to normal, hypophysectomized, gonadectomized, hypophysectomized-tomized or hypophysectomized-thyroidectomized male rats.

#### 15180 P

Hypertension Produced in Dogs by Unilateral Ligation of Periadrenal Blood Vessels and Tissue.

#### JOSEPH VICTOR.

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The possibility that adrenal ischemia may cause hypertension was investigated in the present experiments which are concerned with the effect of decreased adrenal blood supply upon systemic blood pressure.

Method. Mongrel dogs were employed. Systolic blood pressure was determined indirectly by a mercury manometer attached to an inflated cuff wrapped around the hind leg just above the ankle of trained unanesthetized dogs. Systolic pressure was indicated by the pressure at which pulsation of the dorsalis pedis artery was first palpable when the air in the cuff was released. The cuff was inflated 15-30 successive times to determine the range of systolic pressure. These observations were checked by direct measurements with the Hamilton manometer with readings from the femoral artery and showed agreement within 5-15 mm Hg. Animals were housed in separate cages. Blood pressures were recorded daily for a preliminary period of 3-6 weeks. Then either a sham operation in the adrenal area or one to decrease adrenal blood flow was performed on one adrenal. Thereafter blood pressures were taken daily. Urine analysis and blood NPN levels were obtained before and after operation.

The left adrenal was chosen Operation. because it was more easily exposed than the right. Blood vessels come to the left adrenal from the aorta to the hilum, from the diaphragm to the superior pole and from below to the inferior pole. Many arteries enter the adrenal from pericapsular fat and connective tissue. Because of the extreme vascularity of the tissue around the adrenal it seemed that an operation which would constrict or obstruct large numbers of vessels around the capsule would be most favorable for diminishing blood supply. Two ligatures of No. 13 white surgical silk braid were tied about vascular fat and connective tissue adjacent to the adrenal. One was placed around the hilar artery and vein and the other around grossly visible arteries and veins at either the superior or inferior pole. The area involved in the ligature was about 1.0 x 0.5 cm. The procedure just described was used on 5 dogs. In a sixth, No. 391, an artery to the inferior pole and the hilar vein were stripped of fat and connective tissue and ligated with No. 11 surgical silk. To rule out non-specific effects of these procedures, sham operations were performed in 3 dogs, 2 of which had subsequent operations for producing adrenal ischemia. A ligature of

TABLE I.

Hypertension Produced in Dogs by Unilateral Ligation of Periadrenal Tissue and Blood Vessels.

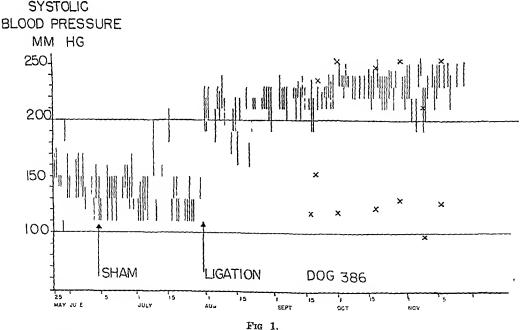
	aysic	mm Hg.	sure,		Died wessen	
Dog No	Before ligation	After sham operation	After ligation	Months after ligation	Blood pressure, r mm Hg. Systohe/diastohe	NPN
361	100 150		180 261	6	261/119	34
384	125 190		190 262	7	248/102	32
385	130 190		195 267	7	267/112	30
386	110 200	130 210	190 253	4	253/130	29
387	120 140	110 150				
390	110 170	130 170	210 280	4	241/131	36
391	130 190		176 255	2	255/108	33

the same material described above was placed through the peritoneum over the surface of the adrenal. Care was taken not to include any grossly visible blood vessels.

Results. Dogs, 4 female and 1 male, subjected to unilateral ligation of periadrenal blood vessels and tissue developed within 1-3 days elevation of blood pressure which has

persisted to the present, 2-7 months later. Another male dog, No. 391, had hypertension after ligation of one artery running to the lower pole of the gland and of the hilar vein. Both systolic and diastolic pressure were elevated in all cases, the latter being over 100 mm Hg. and the former going as high as 280 mm Hg (Table I).

## HYPERTENSION AFTER LIGATION OF TISSUE ADJACENT TO ADRENAL



Vertical lines indicate 1 inges of systolic blood pressures. Upper and lower crosses (X) represent systolic and diastolic pressures respectively. Sham operation on right adrenal had no effect but lightnon of left periadrenal tissues and blood vessels produced hypertension.

Sham operation produced no change in systolic blood pressure in 3 dogs which were followed for 6 weeks. Subsequent operations in which periadrenal blood vessels and tissues were ligated in dog No. 390 and No. 386, Fig. 1, caused elevation of blood pressure which has persisted until the present, 4 months later. To date, there have been no significant changes in the urine analysis or

blood NPN levels of the hypertensive dogs.

Conclusion. A prompt and sustained hypertension was produced in 6 dogs by unilateral subtotal ligation of periadrenal blood vessels and tissue.

It is a pleasure to thank Miss Goldie Spierer and Mr. Dominiek Triolo for their assistance in these observations.

#### 15181

### The Production of Shock in Rats by the Drum Method.*

ROLAND K. MEYER AND ELVA G. SHIPLEY.

From the Department of Zoology, University of Wisconsin, Madison, Wis.

Noble and Collip¹ described a method by which rats were subjected to graded degrees of trauma by tumbling in drums for varying periods of time. Animals subjected to this type of trauma did not develop complications of hemorrhage and infection. The undesirable factor of an anesthetic was likewise eliminated. Hemoconcentration and other pathological changes ascribed to the condition of shock did develop. These workers found that the drum method produced a better graded and controlled type of trauma than any other method they tried, and made use of it in subsequent experiments.2,3 Zahl, Hutner, and Cooper4 used the method and confirmed Noble and Collip's results as to the number of turns, percentage of deaths, survival time, and reproducibility. Chambers, Zweifach, and Lowenstein⁵ employed the method to study circulatory reactions of rats, and Clarke and Cleghorn6 used the drug method to study tissue potassium and phosphorus in shocked rats. In our laboratory we were interested in finding a method for producing shock which would give as nearly uniform and reproducible results as possible in animals to be used for biochemical studies. We wished to determine the chemical changes in the blood and tissues of animals in shock, and in animals which had received sublethal stimuli under conditions which could be readily controlled so that a quantitative relationship between the sublethal and lethal stimuli could be defined.

As the tissues were to be analyzed for extremely labile substances they had to be frozen in situ with liquid air. The necessity for rapid freezing precluded the use of any animal larger than a rat. The method of Noble and Collip for production of shock was chosen because it seemed best to meet the requirements for the chemical investigations and also because it simulates battlefield conditions insofar as it has the elements of violent exercise and excitement as well as trauma.

The blood and tissue studies of rats which had been shocked by the drum method will be reported in other papers. 7.8

This report is concerned with results ob-

- * The work described in this paper was done under a contract, recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of Wisconsin.
- 1 Noble, R. L., and Collip, J. B., Quart. J. Exp. Physiol., and Cog. Med. Sci., 1942, 31, 187.
  - 2 Noble, R. L., and Collip, J. B., abid., 201.
- 3 Noble, R. L., Am. J. Physiol., 1942, 138, 346.
- 4 Zahl, P. A., Hutner, S. H., and Cooper, F. S.,
- J. Pharm. and Exp. Therap., 1943, 77, 143.
  5 Chambers, R. W., Zweifach, B. W., and Lowen-
- stein, B. E., Am. J. Physiol., 1943, 139, 123.
- ⁶ Clarke, A. P. W., and Cleghorn, R. A., Endocrinol., 1942, 31, 597.
- 7 McShan, W. H., Potter, V. R., Goldman, A., Shipley, E. G., and Meyer, R. K., Am. J. Physiol., 1945, 145, 93.
- 8 LePage, G. A., unpublished data.

tained with the Noble-Collip method of producing shock in the Sprague-Dawley strain of albino rats, in a strain of cancer-resistant rats, and in adrenal enucleated rats of the Sprague-Dawley strain.

Materials and Methods. The apparatus used for producing shock was like that described by Noble and Collip except that the drums had a diameter of 16 inches as recommended by the authors. Each of the drums had two baffles and the drums were set to rotate at 40 r.p.m. Metal screen was used to replace the glass in the doors so as to provide better ventilation.

Adult male albino rats of the Sprague-Dawley strain, weighing 250-300 g were used. These rats had been in the laboratory 5 days or longer before being used in any of the experiments. The cancer-resistant strain of rats were those maintained by Dr. M. F. Guyer of the Zoology Department and we are indebted to him for supplying us with them. Males weighing 250-300 g were used.

The adrenal-enucleated rats were Sprague-Dawley rats in which the adrenal capsule had been opened and the cortex and medulla removed by applying light pressure to the capsule. The animals were maintained on 1% NaCl as drinking water for 10 days until the adrenal remnant had regenerated sufficiently to maintain the animals. The rats weighed 125 to 175 g at the time of adrenal enucleation. They were used for the experiment five to six weeks later, at which time the weight ranged from 260 to 360 g. It was obvious that their appetites had been normal and that normal growth had ensued since the average weights were approximately doubled.

Rats were considered to survive if they lived more than 24 hours after tumbling in the drums. To eliminate the possibility of protection from jumping over the baffles the forepaws were fastened together with adhesive tape before the rats were placed in the drums.

Results and Discussion. The animals dying from drum trauma showed essentially the same changes in appearance as described by Noble and Collip.¹ These included extreme vascular dilatation in the intestine and its mesentery, reddening of the muscles of the abdominal wall, frequent hemorrhage into

the lumen of the intestine and petechiæ in the intestinal walls. Lungs sometimes showed either petechiæ or marked congestion. The heart contained little blood and it was thick and viscid. Rarely did an animal show any subcutaneous, cranial, or muscular hemorrhage. Teeth and claws were frequently broken and the feet were often slightly bruised. The liver was engorged and sometimes small breaks and tears occurred. No broken or torn places were ever observed in the spleen. Both adrenals and kidneys often showed congestion.

TABLE I. Survival of Rats to Drum Shock.

No. of rats	Time,* min.	% survival
14	4	100.0
44	8	65.6
42	10	71.4
125	12	47.2
63	13	9.5
16	15	4.3

^{*} Drum revolved 40 times per minute.

The data in Table I show the results obtained by use of 304 male rats of the Sprague-Dawley strain. The per cent of animals surviving is less in almost all cases than was obtained by Noble and Collip at comparable periods of rotation. These results together with ours on conditioning¹¹ indicate that the Sprague-Dawley strain of rats is not as resistant to trauma of this type as are the rats of the strain used by Noble and Collip.

Table II summarizes the results of testing the effect of varying one factor at a time on survival of groups of rats. The first factor which was modified was that of taping all paws in contrast to taping only the front paws as was done regularly when producing Noble-Collip shock. Slightly better survival was obtained when both pairs of paws were taped together. The survival was 42.9% as compared to 21.5% when only the front paws were taped.

Freeman, N. E., Freedman, H., and Miller,
 C. C., Am. J. Physiol., 1941, 131, 545.

¹⁰ Bulbring, E., and Burn, J. H., J. Physiol., 1942, 101, 289.

¹¹ Shipley, E. G., Meyer, R. K., and McShan, W. H., unpublished data.

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The blood and tissue studies of rats which had been shocked by the drum method will be reported in other papers.^{7,8}

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- 6 Clarke, A. P. W., and Cleghorn, R. A., Endocrinol., 1942, 31, 597.
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1.3

shock than are normal animals.2,12,13,14 Numerous investigators have tried the effects of adrenal-cortical extracts as prophylactic and as therapeutic agents in shock produced by various means in both normal and adrenalec-Some of them have found tomized animals the adrenal cortical hormones of benefit 15-17 and others found no beneficial effects from use of these hormones 18-27 In general it may be said prophylaxis with adrenal cortical extracts was found to be of more benefit than therapeusis, and that a greater increase in survival was found in treated adrenalectomized than in treated intact animals. The findings of these authors together with our results in adrenal-enucleated rats suggest that a minimum quantity of adrenal cortical hormones are needed to protect an animal against trauma and that increasing the amount of the extracts is of no further benefit, whereas a decrease in quantity is detrimental.

As a cancer resistant strain of rats was readily available it became of interest to determine whether resistance to cancer might be linked with resistance to trauma. Included in Table II are the results obtained from subjecting 30 adult male rats of the cancerresistant strain to graded degrees of trauma in the Noble-Collip apparatus. The survival at 12 minutes and 10 minutes were respectively 20% and 30%. These results do not seem to show any marked variations from those obtained with the Sprague-Dawley strain, but the percentages of survival are slightly lower.

Summary. 1. Varying degrees of shock can be produced in rats by subjecting them to various periods of trauma in Noble-Collip drums. The method appears to be suitable for use where graded and reproducible degrees of trauma are desired.

- 2. The percentages of survival were. for 5 minutes of tumbling, 100%, for 8 minutes, 65 6%, for 10 minutes, 71 4%, for 12 minutes, 47 2%, for 13 minutes, 9 5%; and for 15 minutes, 43%.
- 3 The Sprague-Dawley strain and a cancerresistant strain of rats were found to be less resistant to trauma of the Noble-Collip type than were rats used by other investigators
- 4 Survival of previous hemorrhage did not increase resistance to Noble-Collip trauma, but appeared to decrease resistance to some extent.
- 5. Adrenal enucleation decreased the resistance of rats to Noble-Collip trauma although regeneration of cortical tissue had been sufficient to support normal growth and to maintain the rats in a healthy state
- 6. Protection to the abdomen and taping of the paws gave only limited protection to the rats of the Sprague-Dawley strain subjected to Noble-Collip shock.

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 $^{^{13}}$  Swingle, W. W., Remington, H. W., Collings, J. W., and Parkins, W. M.,  $\pm m$  J. Physiol., 1941, 132, 249

¹⁴ Hechter, O, Krohn, L, and Harris, J, Endo crinol, 1942, 31, 439

¹⁵ Katz, L. N., Kilhan, S. T., Asher, R., and Perlow, S., Am. J. Physiol., 1942, 137, 79

¹⁶ Weil, P G, Rose, B, and Browne, J S L, Canad Med Assn J, 1940, 43, 8

¹⁷ Shlesei, I. H., and Ashei, R. Am. J. Physiol., 1942, 138, 1

¹⁸ Besser, E L, Arch Surg, 1941, 43, 249

¹⁹ Fine, J., Fischmann, J., and Frank, H. A., Surg., 1942, 12, 1

²⁰ Huizenga, K. A., Biofmin, B. L., and Wiggers, C. J., J. Pharm and Exp. Therap., 1943, 78, 193

²¹ Ingle, D J, Am J Physiol, 1943, 139, 460

²² Keating, F. R., Ji., Power, M. H., and Rynear son, E. H., Current Research in Anosth and Inalg., 1942, 21, 207

²³ Koster, H, and Kasman, L P, Arch Surg, 1942, 45, 272

²⁴ Swingle, W. W., Overman, R. R., Remington, J. W., Kleinberg, W., and Eversole, W. J., Am. J. Physiol., 1943, 139, 481

²⁵ Wigger, C J, Inn Hosp Bull Ann Aibor, 1943, 9, 61

TABLE II.
Alteration in Resistance of Rats to Drum Shock by Various Factors.

No. of rats	Time run,* min.	Factor modified	% survival
14	12	Taping paws—all taped	42.9
14	12	fore paws taped	21.5
19	12	Bled one week previously	5.1
8	12	Without abdominal girdle	0.0
14	12	With	20.0
6	12	Adrenal enucleation	0.0
6	12	Intact adrenals	16.7
24	10	Adrenal enucleation	8.3
12	10	Intact adrenals	50.0
20	12	Cancer resistaut	20.0
10	10	"	30.0

^{*} Drum revolved 40 times per minute.

To determine the effect of protection to the abdomen on survival, 2 groups of rats were run simultaneously in the Noble-Collip drums for 12 minutes. One group was without protection and the second group was protected by having a strip of heavy muslin wrapped snugly around the abdomen of the rat and fastened in place with adhesive tape. The strips were wide enough to cover the space hetween the fore and hind legs and was long enough to make 4 or 5 thicknesses when in place. Some degree of protection against the development of shock was afforded by girdling the abdomen but this protection was not complete, as is indicated by the data in Table II. Chambers, Zweifach, and Lowenstein⁵ found that protection of the abdomen during tumbling prevented the development of a shock-like state in rats subjected to rotation up to 1000 revolutions. Fifty per cent of our rats with abdominal protection survived while none of the controls survived.

Four groups of rats which had survived bleeding to produce hemorrhagic shock were subjected, one week later, to 12 minutes of Noble-Collip shock to see if protection had developed as a consequence of the bleeding. Only 1 rat out of 19 survived the Noble-Collip shock. Comparing the 5.2% survival in these rats with the 47.2% survival in normal rats, it is obvious that a decreased rather than an increased resistance resulted.

The adrenal-enucleated rats were run simultaneously with control rats. Ten or 12-

minute periods of tumbling were used. None of the adrenal-enucleated and only 1 control survived 12 minutes. At 10 minutes 8.33% of 24 adrenal-enucleated and 50% of 12 All of the unconditioned rats survived. adrenal-enucleated rats were autopsied and the regenerated adrenals were removed to determine whether regeneration had occurred. In all cases regeneration of the cortex had occurred. Thus it would seem that loss of the adrenal medulla or perhaps inadequate regeneration of cortical tissue had decreased the percentage of survival. Freeman, Freedman, and Miller7 produced a shock-like state by the infusion of adrenaline. Bülbring and Burn¹⁰ found that small amounts of adrenaline augment transmission in sympathetic ganglia and adrenaline in large amounts depresses transmission. The authors concluded that sudden liberation of large amounts of adrenaline was more likely to produce percirculatory damage than small manent If liberation of adrenaline conamounts. tributes to the production of shock, then the removal of the adrenal medulla should increase the percentage survival in shocked animals, which have been subjected to overwhelming sensory stimuli. However, the results of our experiments do not support this conclusion but it is to be emphasized that the cortex was also largely removed, leaving remnants under the capsule for regeneration. Several investigations have shown that adrenalectomized animals are more susceptible to all types of

TABLE I.
Tumor Takes in Rits Inoculated with Embijo Skin (of the same strain) Prior to Tumoi Implantation.

No. of lats	Latent period, days	Imitial tumor size, mm	Maximum tumor size, uim	Remarks
1	10	8\7\6	35\18\14	Died 24 days following implantation of tumor.
2	10	51414	30×16×14	Died 24 days following tumor transplantation.
3	10	$6 \times 5 \times 4$	33\23\20	77 20 77
4	10	6x6x5	28\20\19	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
5	10	7x6x5	36\25\18	,, ,, ,, ,, ,,
6	12	6x6x4	35\24\20	" 28 " " " "
7	12	4\4\3	38\25\24	11 11 11 11 11
8	12	6x4x4	28\25\29	Killed for tumoi tiansplantatiou
9	12	5x4x4	29\21\18	
10	14	6x5x4	32\26\22	
11	14	4\4\3	28\24\16	All these rats died within 30 to 40 days fol-
12	14	5\5\4	30×25×20	lowing implantation of the tumor.
13	14	61414	28\22\18	
14	14	65554	32\25\19	
15		No growth occ	uned	This lat was resistant to the primary and to subsequent tumor grafts

results as in the previous experiments, ¹⁵ namely the percentage of takes, the latent period of the takes, and the initial and maximum tumor size

In Table I are recorded the observations made on the rats treated with homologous embryo skin prior to tumor grafting and in Table II those of controls. A comparative analysis of the data recorded in these tables reveals the following. Of the 15 experimental rats in Table I 14 developed tumors following a latent period from 10 to 14 days, while among the 6 control rats recorded in Table II all developed tumors following a latent period of 12 days. Judged by the initial and maximum tumor sizes of both experimental and control experiments, the tumors developed at approximately the same rate until the death of the animals

No significance can be attributed to the

fact that one of the 15 rats treated with embryo skin was refractory to the tumor grafts, or to the extended latent period of 14 days among some of these rats (Table I) instead of 10 to 12 days occurring among controls (Table II) in regard to the immunizing effect of the embryo skin. Such single instances were repeatedly observed among control animals of previous experiments. This observation holds particularly true where larger numbers of animals are being used. Rat 15, which proved to be refractory to the first tumor graft, was found likewise to be refractory to subsequent tumor implants. Such a phenomenon has been repeatedly observed among rats of previous experiments. If the first graft fails to produce a tumor, the animal remains refractory to subsequent tumor grafts

It seems safe to conclude, from the ob-

TABLE II.
Tumor Takes in Control Rats of the Same Strain t

- Control Mais of the Game Sciam +						
No of rats	Latent period, days*	Initial tumor size, mini	Max tumoi size, mmt			
1 2 3 4 5	10 10 10 12 12	5\5\4 7\5\4 6\4\4 5\5\4 5\4\4	31×18×15 35×22×17 34×19×15 28×20×16 34×30×19			
6	12	7\5\4	38\29\22			

^{*}Latent period designates time elapsing between implantation of tumor grafts and first appearance of a detectable tumor.

t Maximum tumor size reached at death of animal.

All rats died within 25 to 40 days following implantation of tumor grafts

#### 15182 (

# Failure of Homozygous Embryo Skin to Prevent Growth of Autogenous Tumor-Grafts in the Rat.*

#### ANNA GOLDFEDER.

From the Laboratories of the New York City Cancer Institute and from the Laboratory of Cellular Physiology, Department of Biology, New York University.

A question of considerable interest was brought to my attention on the subject of induced resistance. The question was whether induced resistance can be produced by normal tissues. This concerns the relationship between the immunizing agent and the genetic antecedent of the host. For example, a number of investigators¹⁻¹¹ were able to induce a resistant state to malignant growths including leukemia in hybrid animals by injecting normal homologous tissues, such as liver, spleen, blood, and embryo skin. On the other hand, similar results were not obtained¹²⁻¹⁴ when pure lines of inbred strains of animals were used. From the two sets of observations,

it was concluded that the degree of induced resistance which can be produced depends upon the genetic relationship between the host and the immunizing agent, and that no resistance to tumor growth can be produced either with normal or with malignant tissues in animals of a pure inbred line.

However, the author has reported¹⁵ that there is a possibility of inducing immunity in animals of a pure line to a tumor which originated in the same strain, provided the tumor grafts had been previously attenuated in vitro with specific doses of X-rays.

It was of interest to investigate whether or not the same phenomenon could be demonstrated by the use of normal embryo skin of the same pure inbred strain of rats, for it has been shown^{6,8,11,14} that embryo skin is the most effective agent among normal tissues in this respect.

The technic employed here was similar to that employed by previous investigators14 and consisted of the following: the skin of rat embryos was removed under strictly aseptic conditions, cut with sharp scissors into minute particles and a concentrated suspension prepared in a small amount of 0.85% saline solution. Portions of 0.3 cc of this suspension were injected subcutaneously in the right side of a rat which had been previously shaved and cleaned with alcohol. Twelve days later, the left sides of the same rats were implanted by means of a trocar with grafts weighing about 3-4 mg of the reticulum cell type lymphosarcoma which originated in the same strain. As controls 6 rats of the same strain were implanted with grafts of the same tumor. All the rats of these experiments, both experimental and control, were males. The same criteria were used in the evaluation of the

^{*} This investigation was aided by a grant from the Ella Suchs Plotz Foundation for the Advancement of Scientific Investigation.

Bashford, E., Brit. Med. J., 1906, 11, 209;
 3rd Scient. Rep. Imp. Cancer Fund, Vol. 1904-1908, p. 322.

² Shoene, G., Med. Woch., 1906, 53, 2517.

³ Levine, I., PROC. Soc. EXP. BIOL. AND MED., 1910, 7, 107.

⁴ Lambert, R. A., Proc. Soc. Exp. Biol. and Med., 1911-12, 9, 18.

⁵ Woglom, W. H., J. Exp. Med., Jan., 1910, p. 29.

⁶ Haaland, M., Proc. Royal Soc. London, Series B, 1909-1910, 82, 293.

⁷ Rhoades, C. P., and Miller, D. K., Proc. Soc. Exp. BIOL. AND MED., 1935, 32, 817.

⁸ Kamekura, R., Z. f. Immunitatsforschg., 1925,

⁹ Gardner, R. E., Am. J. Hygiene, 1931, 13, 649.
10 McDowell, E. C., Proc. Nat. Acad. Sci., 1935,
21, 507.

¹¹ Sheever, F. S., Cancer Res., 1941, 1, 23.

¹² McDowell, E. C., Taylor, M. J., and Potter, J. S., Proc. Nat. Acad. Sci., 1935, 21, 507.

¹³ Barrett, M. R., J. Nat. Cancer Inst., 1940,

¹⁴ Eisen, M. J., and Woglom, Wm. H., Cancer Res., 1941, 1, 629.

¹⁵ Goldfeder, Anna, Proc. Soc. Exp. Biol. and Med., 1945, 59, 104.

TABLE I.
Tumor Takes in Rits Inoculated with Embryo Skin (of the same strain) Prior to Tumor Implantation.

No of rats	Latent period, days	Initial tumor size, nim	Maximum tumor size, mm	Remarks	
1	10	81716	35\18\14	Died 24 days following implantation of tumor.	
2	10	51414	30\16\14	Died 24 days following tumor transplantation.	
3	10	6x5x4	33\23\20	" 26 " " " " " " " " " " " " " " " " " "	
4	10	6x6x5	28\20\19	), ), j, i, i, i, i,	
5	10	7x6x5	36x25x18	23 23 23 27 27 27	
6	12	6x6x4	$35 \times 24 \times 20$	,, ₂₈ ,, ,, ,,	
7	12	41413	38x25x24	); ); ); ); ); ); ); ); ); ); ); ); ); )	
8	12	61414	28x25x29	Killed for tumor transplantation	
9	12	51414	29\21\18	1	
10	14	65554	32x26x22	Ì	
11	14	4\4\3	28x24x16	All these rats died within 30 to 40 days fol-	
12	14	5\5\4	30\25\20	lowing implantation of the tumor.	
13	14	61414	28\22\18	) .	
14	14	6x5x4	32\25\19	i	
15	No growth occurred		eurred	This nat was resistant to the primary and to subsequent tumor grafts.	

results as in the previous experiments, 15 namely: the percentage of takes, the latent period of the takes, and the initial and maximum tumor size.

In Table I are recorded the observations made on the rats treated with homologous embryo skin prior to tumor grafting and in Table II those of controls A comparative analysis of the data recorded in these tables reveals the following: Of the 15 experimental rats in Table I 14 developed tumors following a latent period from 10 to 14 days, while among the 6 control rats recorded in Table II all developed tumors following a latent period of 12 days Judged by the initial and maximum tumor sizes of both experimental and control experiments, the tumors developed at approximately the same rate until the death of the animals.

No significance can be attributed to the

fact that one of the 15 rats treated with embryo skin was refractory to the tumor grafts, or to the extended latent period of 14 days among some of these rats (Table I) instead of 10 to 12 days occurring among controls (Table II) in regard to the immunizing effect of the embryo skin. Such single instances were repeatedly observed among control animals of previous experiments. This observation holds particularly true where larger numbers of animals are being used. Rat 15, which proved to be refractory to the first tumor graft, was found likewise to be refractory to subsequent tumor implants. Such a phenomenon has been repeatedly observed among rats of previous experiments. If the first graft fails to produce a tumor, the animal remains refractory to subsequent tumor grafts

It seems safe to conclude, from the ob-

TABLE II.
Tumor Takes in Control Rats of the Same Strain :

		The state of the s		
No. of rats	Latent period, days*	Initial tumor size, mm	Max tumor size, mm†	
1 2 3 4 5	10 10 10 10 12 12	52424 57974 57974 57574	31\18\15 35\22\17 34\19\15 28\20\16 34\30\19	
· · · · · · · · · · · · · · · · · · ·	12	7\5\4	38\29\22	

^{*}Latent period designates time elapsing between implantation of tumor grafts and first appearance of a detectable tumor.

[†] Maximum tumor size reached at death of animal.

[‡] All rats died within 25 to 40 days following implantation of tumoi grafts.

### 15182

# Failure of Homozygous Embryo Skin to Prevent Growth of Autogenous Tumor-Grafts in the Rat.*

#### ANNA GOLDFEDER.

From the Laboratories of the New York City Cancer Institute and from the Laboratory of Cellular Physiology, Department of Biology, New York University.

A question of considerable interest was brought to my attention on the subject of induced resistance. The question was whether induced resistance can be produced by normal tissues. This concerns the relationship between the immunizing agent and the genetic antecedent of the host. For example, a number of investigators¹⁻¹¹ were able to induce a resistant state to malignant growths including leukemia in hybrid animals by injecting normal homologous tissues, such as liver, spleen, blood, and embryo skin. On the other hand, similar results were not obtained¹²⁻¹⁴ when pure lines of inbred strains of animals were used. From the two sets of observations,

it was concluded that the degree of induced resistance which can be produced depends upon the genetic relationship between the host and the immunizing agent, and that no resistance to tumor growth can be produced either with normal or with malignant tissues in animals of a pure inbred line.

However, the author has reported that there is a possibility of inducing immunity in animals of a pure line to a tumor which originated in the same strain, provided the tumor grafts had been previously attenuated in vitro with specific doses of X-rays.

It was of interest to investigate whether or not the same phenomenon could be demonstrated by the use of normal embryo skin of the same pure inbred strain of rats, for it has been shown^{0.8,11,14} that embryo skin is the most effective agent among normal tissues in this respect.

The technic employed here was similar to that employed by previous investigators14 and consisted of the following: the skin of rat embryos was removed under strictly aseptic conditions, cut with sharp scissors into minute particles and a concentrated suspension prepared in a small amount of 0.85% saline solution. Portions of 0.3 cc of this suspension were injected subcutaneously in the right side of a rat which had been previously shaved and cleaned with alcohol. Twelve days later, the left sides of the same rats were implanted by means of a trocar with grafts weighing about 3-4 mg of the reticulum cell type lymphosarcoma which originated in the same strain. As controls 6 rats of the same strain were implanted with grafts of the same tumor. All the rats of these experiments, both experimental and control, were males. The same criteria were used in the evaluation of the

^{*} This investigation was aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.

¹ Bashford, E., Brit. Med. J., 1906, 11, 209; 3rd Scient. Rep. Imp. Cancer Fund, Vol. 1904-1908, p. 322.

² Shoene, G., Med. Woch., 1906, 53, 2517.

³ Levine, I., PROC. Soc. EXP. BIOL. AND MED., 1910, 7, 107.

⁴ Lambert, R. A., PROC. Soc. EXP. BIOL. AND MED., 1911-12, 9, 18.

⁵ Woglom, W. H., J. Exp. Med., Jan., 1910, p. 29.

⁶ Haaland, M., Proc. Royal Soc. London, Series B, 1909-1910, 82, 293.

⁷ Rhoades, C. P., and Miller, D. K., Proc. Soc. Exp. Biol. And Med., 1935, 32, 817.

⁸ Kamekura, R., Z. f. Immunitatsforschg., 1925, 49, 49.

Gardner, R. E., Am. J. Hygiene, 1931, 13, 649.
 McDowell, E. C., Proc. Nat. Acad. Sci., 1935,
 507.

¹¹ Sheever, F. S., Cancer Res., 1941, 1, 23.

¹² McDowell, E. C., Taylor, M. J., and Potter, J. S., Proc. Nat. Acad. Sci., 1935, 21, 507.

¹³ Barrett, M. R., J. Nat. Cancer Inst., 1940,

¹⁴ Eisen, M. J., and Woglom, Wm. H., Cancer Res., 1941, 1, 629.

¹⁵ Goldfeder, Anna, Proc. Soc. Exp. Biol. and Med., 1945, 59, 104.

Noble-Collip¹³ technic.

The purpose of this paper is to report: (1) the method developed for producing graded tourniquet shock, (2) the effect of re-application of tourniquets on the percentage survival in rats, and (3) the hemoconcentration which occurred in tourniquet-shocked rats.

Materials and Methods. The animals used for this study were adult male rats obtained from Sprague-Dawley, Inc. The animals were kept in our laboratory for at least 5 days before being used, at which time they weighed 250-325 g. They were maintained on the regular stock diet and tap water ad libitum.

The tourniquets used were designed so that a set screw could be adjusted against a metal bar in such a way as to tighten a loop of cord slipped over the leg of the rat. The metal portions of the tourniquets were of brass and the cord was ordinary fish line. The tourniquets were applied to both hind legs as high on the thighs as possible and tightened until the screws could no longer be turned by the normal amount of force applied by the fingers. When this was accomplished the muscles of the legs were rigid and the circulation was occluded as shown by cyanosis. The tourniquets were left in position for varying periods of time, depending upon the degree of shock desired.

The rats were anesthetized by Nembutal (Abbott Laboratories) given intraperitoneally just before the tourniquets were adjusted. The usual dose required was 0.2 cc. During the time the tourniquets were in place the animals were given additional amounts (0.05-0.10 cc) of Nembutal as needed to keep them in a semi-conscious state, but the anesthetic was not given after the tourniquets were removed.

As an indication of the course of hemoconcentration occurring after the removal of the tourniquets, hemoglobin determinations were made on tourniquet-shocked rats by the method of Evelyn¹⁴ for oxyhemoglobin. Blood for these studies was obtained by cutting off the tip of the tail. Results and Discussion. Survival after tourniquet shock: The percentages of animals surviving application of tourniquets for various periods of time are summarized in Table I.

TABLE I.
Survival of Rats Subjected to Tourniquets for
Varying Periods of Time.

No. of rats	Tourniq	uet applied	% survival
	lır	min	
7	3	30	100
8	3	35	75
15	3	40	47
12	3	45	33.3
33	4	a	3.0
4	4	15	0
. 6	4	30	Ø
4	8	0	0*

^{*} None of these rats survived more than 3 hours after removal of the tourniquets.

It can be seen that the number of animals surviving decreased as the period of tourniquet application increased. The exact length of the survival period was not determined for every animal that died since some animals died during the night. The usual period of survival was 6 to 18 hours after removal of the tourniquets, but there seemed to be no correlation between the length of the period that the tourniquets were in place and the length of the survival period in those animals which died, except that when the tourniquets were applied for 8 hours the rats all died within 3 hours after removal of the tourniquets (Table I). The usual course of events after tourniquet removal was a period of approximately 4 or 5 hours in which the animal seemed to be in good condition, in spite of the fact that the legs showed increasing edema. The circulation was restored soon after the release of the tourniquets except in an occasional rat in which the vessels to the limb were permanently occluded as indicated by the continued cyanosis of the limb and the absence of edema. In some cases cyanosis remained yet edema developed. This could be due to venous occlusion or loss of whole blood into the traumatized area. During the first 1 or 2 hours after release the legs showed the greatest increase in size, but they continued to become gradually larger during the course of the first 5 or 6 hours. During that period fluid was lost into the tissue adjacent to the tourni-

¹³ Noble, R. L., and Collip, J. B., Quart. J. Exp. Physiol. and Cog. Med. Sci., 1941, 31, 201.

¹⁴ Evelyn, K. A., J. Biol. Chem., 1936, 115, 63.

servations recorded in the Tables I and II, that inoculations of embryo skin into rats of the same genetic constitution, fail to elicit a resistant state to grafts of a tumor autogenous to the strain. This observation is in accord with findings of previous investigators who likewise failed to elicit immunity in a pure line of animals with embryo skin of the same genetic line.¹⁴

Discussion. There is no intention here to discuss generally the genetic principles involved in the problem of induced resistance to malignant growth. As indicated in the previous communication¹⁵ the results obtained are specifically concerned with the reticulum cell type lymphosarcoma, originating in a pure line of rats of Dr. Bagg. It was suggested that the type of tumor may be responsible for the specific inimune action in an

inbred strain of rats. The results obtained from the experiment described in the present paper brought further evidence, in this case, that the induced resistance is specific to the type of tumor and independent of the genetic inter-relation between the host and the immunizing agent, inasmuch as embryo skin of the same strain proved to be ineffective in eliciting the same phenomenon.

On the other hand the results of the present paper are in full agreement with those obtained by other investigators, showing that no resistance to tumor grafts can be elicited in animals of a pure line with embryo skin of the same strain.

Summary. Rats of a pure inbred line treated with embryo skin of the same strain failed to produce a resistant state to grafts of a tumor autogenous to the strain.

#### 15183

Shock Produced by the Application of Tourniquets to the Hind Limbs of Rats.*

ELVA G SHIPLEY, ROLAND K MEYER, AND W. H. McSHAN. From the Department of Zoology, University of Wisconsin, Madison, Wis

The tourniquet method has been accepted as a valid means of producing experimental shock and numerous investigators have used some variety of tourniquets on laboratory animals, including albino rats. Some investigators have used rubber bands, 1.2 cord tied tightly or wrapped in numerous turns

about the leg, ¹⁸ and compression from clamp devices to cut off the circulation to a limb ^{9,10} We were interested in standardizing the tourniquet method so that graded and reproducible degrees of trauma could be effected Animals shocked by this method were to be used in making biochemical studies for comparison with similar studies¹¹ ¹² on rats shocked by the

^{*} The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Wisconsin

¹ Allen, F. M., Am. J. Surg., 1943, 60, 335

² Allen F. M., Arch Phys Ther., 1943, 24, 327

³ Ingle, D J. Am. J Physiol, 1943, 139, 460

Katzenstein, R., Mylon, E., and Winternitz, M. C., Am. J. Physiol., 1943, 139, 307

⁵ Kleinberg, W., Remington J. W., Eversole, W. J., Overman, R. R., and Swingle, W. W., Am. J. Physiol., 1943, 140, 197.

⁶ Mylon, E, Winternitz, M C, and de Suto Nagy, G. J., Am J Physiol, 1943, 139, 313

⁷ Mylon, E, Winternitz, M. C, Katzenstein, R, and de Suto Nagy, G. J., Am. J. Physiol., 1942, 137, 280

⁸ Swingle, W. W., Overman, R. R. Remington, J. W., Kleinberg, W., and Eversole, W. J., Am. J. Physiol., 1943, 139, 481

⁹ Green, H. N., Camb, M. A., and Sheff, M. SC., Lancet, 1943, 11, 147

¹⁰ Haist, R E, and Hamilton, J. I, J Physiol, 1944, 102, 471

¹¹ LePage, G A, unpublished data.

¹² McShan, W. H., Potter, V. R., Goldman, A., Shipley, E. G., and Meyer, R. K., Am. J. Physiol., 1945, 125, 93

TABLE II.
Survival of Rats After Reapplication of Tourniquets Following Varying Periods of Release.

No. of rats	Tourniquet applied	Tourniquet released	Tourniquet reapplied	% surviva
	hr	hr	hrs	
6	7	1	Through 24	100
6	4	2	", 24	100
6	4	3	" 24	100
6	4	4	" 24	100
8	4	5	" 24	100
8	4	6	,, 2 <del>1</del>	100
8	7	7	", 24	87.5

concentration in animals at the time of death would preclude the use of it as an indication that the irreversible stage had been reached in tourniquet shock.

Reapplication of tourniquets: Experiments were designed to find the stage at which the irreversible condition is reached in tourniquet shock, and to determine if it is the period of ischemia alone or some subsequent change that is the decisive factor in this type of shock. Adult male rats were subjected to tourniquets for 4 hours, then the tourniquets were released for periods varying from 1 to 7 hours, then replaced and left in position through 24 hours from the time of first release. The rats were lightly anesthetized with ether for the replacement of the tourniquets, but they were not further anesthetized during the period of reapplication. The tourniquets were then removed and the rats were observed for the next 24 hours to determine survival rates. The data in Table II summarize the results of this experiment and by comparison with those in Table I it can be seen that only 3% of the rats survived 4 hours of tourniquets when there was no replacement, but in the case of temporary release for 1 to 6 hours 100% of the animals survived during the following 24 hours of reapplication. tourniquets were then released and the animals were observed for an additional 24 hours. Two animals died after the second release of the tourniquet. Death in one of these rats followed severe hemorrhage from the traumatized tissue. The rats were regularly sacrificed after 48 hours because the limbs were necrotic. After replacement of tourniquets the legs had become cyanotic and remained so after the tourniquets were released. The rats were otherwise alert and energetic. When the release period was increased to 7 hours 87.5% of the animals survived when tourniquets were reapplied. The irreversible stage of shock was probably prevented from developing by the elimination of circulation to the injured limbs and the prevention of any further loss of fluid into the injured area and the escape of metabolites from the injured tissue. Thus the animal could successfully cope with the first loss of fluid and the first of the metabolites from the injured area. Green et al.9 found that after clamping both legs of rats for 7 hours removal of limbs up to 21/2 hours saved all animals, but "from 21/2 hours onward the survival rates steadily fell, to reach zero, on the average, at 4 hours." They further observed that the rat could withstand repeated sublethal doses of clamping totalling at least 12 out of 32 hours.

It would appear that local fluid loss to the limb after release of the tourniquets cannot be the only factor responsible for the development of irreversible shock as reapplication of the tourniquets after fluid loss has occurred prevents death. The importance of fluid loss in decreasing the ability of the animal to recuperate cannot be denied, but it is to be emphasized, as blood12 and muscle analyses show,11 that after tourniquet removal substances from the limb escape into the circulation. The accumulation of these metabolic breakdown products probably interferes with cellular metabolism and damage to vital organs results. The beneficial effect following reapplication of tourniquets suggests that the total quantity of the injury substances, as well as the length of time over which they act, is of importance in determining the fate of the animal.

Summary. 1. A method for producing

TABLE III.									
Hemoglobin	Concentration	in	Rats	in	Tourniquet	Shock.*			

	1† Hb-before	2 Hb– after	3	4	5		
No. of rats	tq. removal	tq. removal	Hb- increase	Hb~last sample	Hb- increase	Survival +/	Hour of death
	%	%	%	%	%		
1	84.0	111.5	27.5	111.5	27.5		7
2	85.0	137.2	52.2	137.2	52.2		7
3	92.0	126.4	34.4	126.4	34.4		8
4	79.0	117.3	38.3	117.3	38.3		4
5	98.1	130.1	32.0	121.8	23.7	Sacrificed	5
6	81.6	126.4	44.8	118.6	37.0	,,	5
$\Lambda vg$	86.6	124.8	38.2	122.1	35.5		

* 100% hemoglobin equals 15.6 grams per 100 cc.

quet-traumatized tissue, and into the genital region, particularly the scrotum. After these first hours changes in gross size could not be detected by observation. Haist and Hamilton¹⁰ found that the swelling of the limb reached its maximum during the second hour after removal of the clamps, but in their rats the clamps had been left on overnight (12-15 hrs) and the average survival time was only 3 hours 12 minutes. It might be expected that maximum edema would be reached more rapidly after longer periods of tourniquets.

Hemoconcentration in tourniquet shock: Hemoconcentration is usually an accompaniment of shock and it was of interest to follow the course and extent of hemoconcentrations in normal rats during the period following tourniquet removal when shock was developing. Hemoconcentration may be determined by measurement of hematocrit, by increase of specific gravity, by hemoglobin determinations and by red cell counts. In our laboratory hemoglobin determination was the most feasible method to use as an index of hemoconcentration.

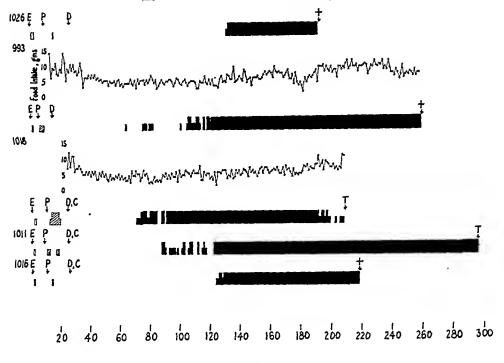
It is known that splenic contraction occurs in shock, and that it contributes to hemoconcentration. Taylor and Page, 15 using dogs, found that "the splenic contribution to changes in the hematocrit index occurs during the first one or two hours of the experiment." The authors state that splenectomy abolishes the

early sharp rise of the hematocrit index seen in shock. On the basis of these studies it is probable that the early increase in hemoglobin observed after the removal of tourniquets can be ascribed only in part to fluid loss. This fact in no sense invalidates the use of hemoglobin determinations as indications of hemoconcentration since we were interested in relative rises in hemoconcentration extending over a number of hours.

Hemoglobin determinations were made on rats which had tourniquets in place for 4 The first determinations were made at the time of the removal of the tourniquets, and each hour thereafter for periods as long as 7 hours. Two of the animals for which data are given were sacrificed at the end of 5 hours to obtain blood for analyses to be reported in connection with other blood studies.12 Table III is a summary of the hemoglobin values found during the period after the tourniquets were removed, and shows the highest concentration found for each of the animals during the experimental periods, that found in the last sample, and the increases of each of these over the original concentration. In the shocked rats hemoconcentration developed progressively after the removal of the tourniquets until the death of the animal. Increases in hemoglobin in rats which were followed to the hour of death ranged between 27 and 52%. From these data the conclusion can be drawn that marked hemoconcentration is an accompaniment of shock produced by tourniquets and that the wide variation in degree of hemo-

t The values in column 3 were obtained by subtracting the values in column 1 from those in 2; the values in column 5 by subtracting those in 1, from the ones in 4.

¹⁵ Taylor, R. D., and Page, I. H., Arch. Surg., 1943, 47, 59.



DAYS

Series of rats receiving a diet low in proteins and high in fat. (5 of 9 rats in Group A). The blocked areas represent estrus. Partial estrus is depicted by a reduction in height of the block. Symbols are as follows: E—Test dose of estrone, P—Insertion of pellet, D—Diet started, C—Supplement of cystine, T—Experiment terminated. +, death of animal. The tats were castrated at zero time.

fed a diet low in lipotropic factors.

Reversal of the estrus reaction by the administration of large doses of yeast indicates that curative measures for dietary hepatic injury may be adequately assessed in the living animal.

Yeast as a curative factor in dietary hepatic injury is in good accord with previous findings obtained in prophylactic experiments of long duration (150 days). Addition of yeast to the basal experimental diet prevented the production of hepatic injury as controlled by macroscopic and microscopic examinations of the liver. However, yeast is not the best and most characteristic representative of the lipotropic substances. Given as sole source of protein and not—as in our previous

studies—as supplement to a diet with a low casein content as source of protein, yeast may even support dietary hepatic injury, especially necrosis.⁶ Furthermore, yeast is rich in vitamins of the B group and in many other cell constituents. Thus, curative effect exerted by yeast has to be considered too complex to permit detailed analysis. Judging from prophylactic studies based on pathological findings in the dead animal a curative effect similar to that of yeast might be logically anticipated from simple substances such as methionine or methionine-containing proteins, for example casein or lactalbumin. In contrast, even manifold increased vitamin B supple-

 ⁵ György, P., and Goldblatt, H., J. Exp. Med.,
 1942, 75. 355. Also György, P., Am. J. Clin. Path.,
 1944, 14, 67, with bibliography.

⁶ Hock, A., and Fink, H., Z. f. physiol. Chem.,
1943, 278, 136, and 1943, 279, 187; Himsworth,
H. P., and Glynn, L. E., Lancet, 1944, 1, 457;
Glynn, L. E., and Himsworth, H. P., J. Path. and
Bact., 1944, 56, 297.

tourniquet shock in rats is described. The method yields graded and reproducible results.

- 2. The percentage survival of rats to tourniquet shock was progressively decreased as the time of tourniquet application was increased. Following 3½ hours of tourniquets 100% of the rats survived, while after 4 hours of tourniquets only 3% survived.
- 3. Rats which had tourniquets applied for 4 hours with periods of release for 1 to 6

hours, followed by a second application of tourniquets, showed 100% in contrast to 3% survival for those without replacement, while 87.5% survived when the tourniquets were replaced after 7 hours.

4. The degree of hemoconcentration developing in rats in tourniquet shock was approximated by hemoglobin determinations. Increases in hemoglobin ranged between 27 and 52%.

#### 15184

Inactivation of Estrone by Liver. Assay Method In vivo for Dietary Hepatic Injury in Rats.*

#### PAUL GYÖRGY.

From the Department of Pediatries, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Pellets of estrone implanted in the spleen will not induce estrus in ovariectomized rats fed a normal diet.1 Estrone draining from the pellet through the splenic vein into the liver is inactivated by the normal functioning liver parenchyma, either through direct chemical destruction1 or through a closed "hepatointestinal cycle."2 Failure of the liver to inactivate estrogen has been demonstrated by Biskind and Biskind1 in rats maintained on a diet deficient in the vitamin B complex. Estrus in such animals appeared within 1 to 3 weeks and was interpreted as an indication of Such functional impaired liver function. change resulting from avitaminosis is in its etiology and pathogenesis different from the specific hepatic injury (necrosis and cirrhosis) produced in rats by the administration of a synthetic diet low in lipotropic factors (methionine and choline),3 but containing all known vitamins including the members of the vitamin B complex. On the other hand, however, it has been shown4 that with regard to inactivation of estrogen by the liver, rations deficient in lipotropic factors will have the same effect as rations free of vitamin B complex. In spite of this analogy only the disturbance elicited by rations deficient in lipotropic factors with its characteristic pathological manifestations is considered the true dietary hepatic injury.³

In previous experiments⁴ the onset of functional disability of the liver, measured by the failure of inactivation of estrone, became evident in rats fed a diet low in lipotropic factors after an interval when severe histological changes are known to make their appearance. This interval was much longer than that observed in rats fed a vitamin B-free diet.^{1,4} Whereas the impaired inactivation of estrone in rats kept on a vitamin B-free diet could be reversed and normalized by supplements of vitamin B complex,^{1,4} large doses of yeast were needed to achieve the same result in rats

^{*} Aided by a grant from Wyeth, Incorporated, Philadelphia.

¹ Biskind, M. S., and Biskind, G. R., Endocrinol., 1942, 31, 109. Here also literature.

² Cantarow, A., Paschkis, K. E., Rakoff, A. E., and Hansen, L. P., Endocrinol., 1943, 33, 309.

³ György, P., and Goldblatt, H., Proc. Soc. Exp. Biol. AND Med., 1941, 46, 492; Webster, G., J. Clin. Invest., 1941, 20, 440; Blumberg, H., and McCollum, E. V., Science, 1941, 93, 598; Lillie. R. D., Daft, F. S., and Sebrell, W. H., Pub. Health Rep., 1941, 56, 1255.

⁴ Shipley, R. A., and Gyorgy, P., Proc. Soc. Exp. Biol. and Med., 1944, 57, 52.

tion of cirrhotic changes in the liver.5

Results. The appearance of estrus showed great variations. It is remarkable that 35 out of a total of 91 experimental animals remained in anestrus for the whole duration of observation, up to 150 days or more. Two rats showed only temporary or irregular estrus. In the remaining group of 54 rats regular estrus was observed 39 to 195 days, with a calculated average of 86 days after the animals were put on the basal experimental diet. In several instances estrus when first noticed showed some fluctuation before going into solidly sustained complete estrus (Fig. 1, rats 993 and 1011).

On the assumption that normal liver "inactivates" estrone, lack or retarded appearance of estrus should indicate absence or delayed production of hepatic injury with its characteristic pathological attributes. Such behavior would be at variance with the reactivity of normal rats³ and could only be explained by the presence of estrone pellets in the spleen preceded by ovariectomy in the present experimental group of animals.§

Rats showing the appearance of continuous estrus were kept under observation for 2-4 weeks without any change in the experimental conditions. At the end of this preliminary period the animals were divided in 5 subgroups (A to E). Subgroup A was kept as control on the unchanged original regime. Rats in B received dl-methioninell in daily doses of 50 mg, in C a digest of casein (Amigen) 1 g daily, in D a digest of lactalbumin (Lactamin) 1 g daily. In subgroup E the daily basal vitamin supplements were increased fivefold. In instances when cystine was given before the appearance of regular estrus, it was discontinued as soon as treatment with lipotropic factors (methionine or protein digests) started.

Once established, full estrus persisted throughout the total period of observation in Group A (9 animals), in which the original experimental conditions remained unchanged, and in Group E (13 rats) in which the

animals received a fivefold increase in the amount of basal vitamin B supplements. The solid curve of full estrus in such animals (See Fig. 1 and 2 with representative examples) served also as control for experiments in which estrus has disappeared under the influence of special curative measures. Of course, even without special treatment estrus, when artificially supported by a pellet of estrone implanted in the spleen, cannot last permanently and must cease as soon as the pellet is completely absorbed or becomes tightly encapsulated (Rat No. 1018 in Fig. 1). This last possibility has to be borne in mind in cases where estrus was seemingly suppressed after application of special curative measures. order to avoid false conclusions a positive result should not be considered as such unless with discontinued therapy estrus reappears, thus proving the presence of properly functioning estrone pellets.

This special experimental procedure was fully observed in subgroups B, C, and D in which the therapeutic effect of methionine (16 animals), casein-digest (11 animals), and lactalbumin-digest (5 animals) was investigated. The results have shown unequivocally that methionine (Fig. 3) as well as the 2 protein-digests (representative examples for lactalbumin in Fig. 2) exerted a definite therapeutic effect which manifested itself in complete or at least in partial suppression of estrus. Interruption of treatment was followed in due course by reappearance of full estrus. The time required for the suppression of estrus by means of supplements of lipotropic factors showed considerable variation, ranging from a few days to 10 weeks, with an average of 31 days.

In a number of rats (in all subgroups) daily food intake was measured. No correlation could be found between appearance or disappearance of estrus and the curve of daily food intake.

Discussion. The assumption submitted previously that impairment of estrone inactivation by the liver observed in rats on a cirrhosis-producing diet resulted from the specific dietary hepatic injury, is well borne out by the present studies. Fivefold increase in the amount of daily vitamin B supplements

[§] This special problem will be discussed in a separate communication. Preliminary report appeared in Federation Proceed., 1945, 4, 155.

[|] Kindly furnished by Wyeth, Incorporated.

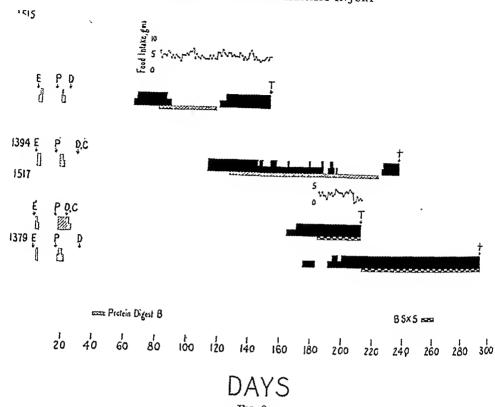


Fig. 2.

The effect of protein digest—lactalbumin digest (in 2 of 5 rats in Group D) and of fivefold vitamin B supplements (in 2 of 13 rats in Group E). The symbols have the same meaning as in Fig. 1.

ments should prove to be devoid of any curative effect.

The following experiments deal mainly with these special questions. They were undertaken to produce supporting evidence for the usefulness of the method of estrus reaction in the study of experimental dietary hepatic injury in the living animal.

Methods. Rats used were of 3 separate strains: 1. Sprague-Dawley (65 rats), 2. Wistar (9 rats), and 3. a strain obtained from a local dealer (17 rats). Animals were selected with a weight of 150-230 g, the majority weighing approximately 200 g. The preparation of the animals for the experiments, including surgical technic, was the same in the previous study. The experimental diet was slightly modified, as follows: Casein (SMACO) 8, Crisco 38 (or, in some experiments, Crisco 30 and peanut oil 8), sucrose 50, salt mixture 4. The vitamin sup-

plements were given separately: (a) Solution of B vitamins (1 cc daily) containing thiamine 20 y, pyridoxine 20 y, riboflavin 25 y, Ca-pantothenate 100 y. (b) Vitamins A and D in form of Ol. percomorph., 3 drops weekly. (c) Vitamin E as special supplement was only used in the experiments with the local and Wistar strains (3 mg in the form of natural concentrate once a week). In the remaining experiments the vitamin E requirement was covered solely by the basal diet with its high content of Crisco, a fat rich in vitamin E.: More than half of the experimental animals received as a further separate supplement 50 mg of *l*-cystine daily. The purpose of this supplement was the acceleration and aggrava-

t Kindly furnished by Distillation Products, Inc., Rochester, N.Y.

^{*} According to a personal communication of Dr. K. E. Mason (Rochester), 5% of Crisco in the diet represents the minimal protective level.

diet. This explanation, however, disregards the important fact that estrus disappeared even in cirrhotic rats as soon as the estrone pellet was completely absorbed. Lack of estrone should not influence the pseudo-estrus of vitamin A deficiency.

Summary. Impairment in ability to inactivate estrone accompanies dietary hepatic

injury and can be rectified by the addition of lipotropic factors such as methionine or protein digests.

Fivefold increase of the basal vitamin B supplements was ineffective.

The author is greatly indebted to Mrs. C. K. Gilkey for technical assistance.

#### 15185

Studies on Toxicity Complement-Fixing and Immunogenic Activity of Typhus-Infected Yolk Sacs.

VINCENT GROUPÉ AND RICHARD DONOVICK.* (Introduced by Geoffrey Rake.)

From the Biological and Chemical Laboratories, E. R. Squibb & Sons, New Brunswick, N.J.

The use of the yolk sac of the developing chick embryo for cultivating typhus rickettsiæ¹ led to the development of vaccines prepared from infected yolk sacs by phenol precipitation² and ether extraction³,⁴ and to the demonstration of a labile toxic substance in yolk sac cultures of typhus rickettsiæ which was lethal for mice and which was associated with the rickettsiæ themselves.⁵,6

It has been shown that both the complement-fixing activity and the rickettsial content of typhus vaccines were related to the time of harvest of the constituent yolk sacs with reference to the peak of embryo deaths. However, it was also observed that the presence

of numerous rickettsiæ did not necessarily indicate toxicity⁰ and that the antibody response of guinea pigs, commonly used as a measure of antigenicity, is subject to considerable variations.⁸ It was of interest, therefore, to compare complement-fixing activity with the toxicity of yolk sac suspensions and with the antigenicity and rickettsial content of typhus vaccines.

Methods. Eggs in the 7th day of embryonic development were inoculated into the yolk sac with the Breinl strain of epidemic typhus according to the method of Cox.¹ The eggs were candled daily and all yolk sacs harvested were frozen immediately and stored at -70°C until used. All vaccines were prepared by the ether extraction method¹ using a standard procedure (cf. ⁷).

Complement-fixation tests were done according to the method of Bengtson. Vaccines were titrated with 2 units of hyperimmune guinea pig serum and the titer was recorded as the highest dilution of vaccine showing complete (4+) fixation.

Toxicity titrations were performed as follows: 0.5 ml of serial 2-fold dilutions of the various yolk sac suspensions was injected into

^{*}On temporary leave from the Division of Microbiology, The Squibb Institute for Medical Research.

¹ Cox, H. R., Pub. Health Rep., 1938, 53, 2241.

² Cox, H. R., Science, 1941, 94, 399.

³ Connaught Laboratories, Toronto, Ontario, War Project Med. 8, November 13, 1942 (N.R.C. Canada). Memorandum No. 3.

⁴ Topping, N. H., National Institute of Health, Washington, D.C., 1945, Bull. No. 183.

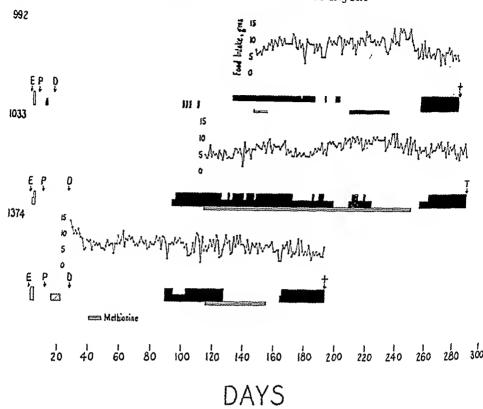
⁵ Gildemeister, E., and Haagen, E., Deut. Med. Wchnschr., 1940, 66, 878.

⁶ Bengtson, I. A., Topping, N. H., and Henderson, R. G., Nat. Inst. of Health, Washington, D.C., 1945, Bull, 183.

⁷ Groupé, V., Nigg, C., and MacFarlane, J. O., J. Immunol., in press.

⁸ Donovick, R., Farrell, M., and Smith, F., J. Bact., 1945, 50, 241.

⁹ Bengtson, I. A., Pub. Health Rep., 1941, 56, 649.



The effect of methionine (in 3 of 16 rats in Group B). The symbols have the same meaning as in Fig. 1 and Fig. 2.

had no effect on estrus appearing in this group of rats. Thus it can be denied that the liver injury produced by cirrhosis-producing diet has led to impairment of estrone inactivation solely by increasing the need for the B vitamins. Such relative vitamin B deficiency would have been the counterpart of the similar effect of absolute lack of B vitamins.

With the demonstration of the therapeutic effect exerted by lipotropic factors such as methionine or methionine-containing protein digests (casein and lactalbumin) the specific character of the impairment of estrone inactivation in this group of rats is well substantiated. The same lipotropic factors play a leading part in the prevention of dietary hepatic injury, proven by macroscopic and microscopic evidence. The present observations should arouse special interest for two reasons: 1. They were made on living animals, and 2. They indicate strongly the possibility

of therapeutic reversal of the underlying hepatic changes, in contrast to past routine studies of prophylactic nature. Inasmuch as treatment was initiated soon after the functional hepatic disturbance became apparent, it is improbable that major fibrotic changes in the liver could have been developed before therapy was started. In consequence, the restored suppression of estrus should not be construed as an outward sign of completely Morphological cure restored architecture. may be deduced only from direct histological examination. On the other hand the methods of estrone inactivation could become useful in the evaluation of possible therapeutic factors on living animals suffering from dietary liver It is conceivable to regard estrus appearing in rats fed a cirrhosis-producing diet as the equivalent of pseudo-estrus of vitamin A deficiency, based on the inability of cirrhotic livers to utilize vitamin A of the

TABLE II.

Comparison of Complement-Fixing Activity, Rickettsial Content and Antigenicity of Vaccines Prepared from Embryos Harvested Before and at the Peak of Embryo Deaths.

		Vaccines pr harvest D 5				
No. of yolk saes included in vace Rickettsial content		90 ± 3+	102 +	146 ++	65 ++++	
Complement fixation test	Vaccine diluted.18 1 16 1 32 1 64 1 128	3+ 1+ ± 0	4+ 3+ 2+ 0	4+ 4+ 3+	4+ 4+ 4+ 1	
Antigenicity for mice Method A-0.2 ml undiluted inoculated I.P. Vaccinated group Total No. of	vaccine	28	27	Ü	28	
No protected % protected Control group Cha	allenge dose diluted:12		17/27 62 9	n.t.	24/28 85.7	0/16* 10/15,
Method B-0 5 ml of serial of vaccine inoculated I.P. Vaccinated group	Vaccine diluted: 15 125 1 125	0/9 0/10	4/9 0/10	n.t.	7/10 6/10	
Control group Cha	allenge dose diluted:12	0/10	0/10		0/10	0/10 8/10

D 5 Dead 5 days after moculation.

of the mice immunized with vaccine L-7 were When mice were immunized by Method B vaccine D-5 diluted 1-5 did not protect any of the 9 vaccinated mice whereas vaccine D-6 diluted 1-5 protected 4 of 9 vaccinated mice, and vaccine L-7 diluted 1-25 protected 6 of 10 vaccinated mice. In addition to the data presented, in 7 additional experiments the complement-fixing activity of the vaccine likewise followed the antigenicity of the vaccine.

Discussion. It is clear from the data presented that complement-fixing activity followed not only the rickettsial content and antigenicity for mice of vaccines prepared in an identical manner but also the toxicity of yolk sac suspensions when care was taken to preserve toxicity. However, the factors responsible for toxicity and complement-fixing activity are not identical since toxicity is destroyed by formalin and ether6 while complement-fixing activity is not. Furthermore, Complete fixation of complement.

0 No fixation of complement.

the complement-fixing activity of a given yolk sac suspension or vaccine represents not only the rickettsiæ themselves but also a soluble antigen capable of immunizing guinea pigs.10 Nevertheless, when yolk sac suspensions or vaccines were prepared as described from embryos harvested on consecutive days an increase in complement-fixing activity was always accompanied by an increase in toxicity or antigenicity.

Summary. Under the conditions described, the toxicity of untreated yolk suspensions and the antigenicity of typhus vaccines prepared from embryos harvested on consecutive days rose together with the complement-fixing activity of the same antigens.

The authors wish to acknowledge the technical assistance of Mr. Jack O. Davis.

L-7 Living 7 days after inoculation. + Five rickettsiae per field.

⁺⁺⁺⁺ Over 100 rickettsiae per field.

n.t. Not tested.

^{*} No. of mice surviving/Total No. of mice.

I.P. Intraperitoneally.

¹⁰ Topping, N. H., and Shear, M. J., Pub. Health Rep., 1944, 59, 1671.

Comparison of Toxicity with	TABLE I.			
proof but	Suspensions.*	Activity o	f Infected	Yolk Sac
rval between				

noculation and harvest	No. of yolk saes represented	Comple suspe	usion di	Toxicity titration		
L J		1-16	1-32	1-64	suspen:	sion dilute
L-6	15	0	0			4 1-8 1-
L-7	15	3+	1+	U,	0/31 0/-	0/4
L-8	15	44	2+	* *	4/4 4/4	0/4 0/

No. of mice dead/ Total No. of mice.

the tail vein of mice.† The number of deaths was recorded 18 hours after inoculation.

The approximate rickettsial content of a vaccine was estimated by microscopic examination of films of vaccine made with a standard loop and stained by Macchiavello's technic

Antigenicity was measured by immunizing mice as follows: Method A: Each of 30 mice was given one intraperitoneal inoculation of 0.2 ml of undiluted vaccine. Method B: Groups of 10 mice each were given one intraperitoneal inoculation of 0.5 ml of vaccine diluted 1-5, 1-25, and 1-125 respectively. Fourteen days after vaccination all vaccinated mice were challenged with 0.5 ml of toxic substance representing 2-4 LD50 injected into the tail vein. The results were recorded 18 hours after challenge.

Experimental. Comparison of toxicity with complement-fixing activity of infected yolk sac suspensions. Inasmuch as both the rickettsial content and complement-fixing activity of vaccines were highest when the constituent yolk sacs were harvested from embryos still living at the peak of embryo deaths,7 yolk sacs were harvested only from living embryos to prevent loss of toxicity following death of the embryo. In comparing toxicity with complement-fixing activity yolk sacs were harvested from 15 living embryos on the 5th, 6th, 7th, and 8th day after inoculation respectively. Each pool of yolk sacs thus obtained was frozen immediately and stored at -70°C. Approximately one week later 10% untreated tissue suspen-

sions in saline were prepared from each poor of yolk sacs and each suspension was titrated for complement-fixing activity and for toxicity in mice. It will be seen from Table I that the complement-fixing titer of yolk sac suspensions increased from less than 1-16 in the case of suspension L-5, prepared from embryos harvested 5 days after inoculation, to 1-32 in the case of suspension L-8, prepared from embryos harvested 8 days after inoculation. When the same suspensions were titrated for the presence of toxic substance by intravenous injection of mice suspension L-5 diluted 1-2 was not lethal for mice whereas suspension L-8 diluted 1-8 was lethal for all mice inoculated. It is thus evident that the complement-fixing activity and toxicity of untreated saline suspensions of infected yolk sacs harvested from living embryos rose together.

Comparison of antigenicity with complement-fixing activity and rickettsial content of typhus vaccines. The complement-fixing activity, rickettsial content and antigenicity of typhus vaccines prepared from yolk sacs harvested before and at the peak of embryo deaths were compared and the results are presented in Table II. It was found that the complement-fixing titer increased from less than 1-8 in the case of vaccine D-5, prepared from embryos dead 5 days after inoculation, to 1-64 in the case of vaccine L-7, prepared from embryos still living 7 days after inoculation and that the rickettsial content of the vaccines increased with the complement-fixing When mice were immunized by Method A only 6 of 28 (21.4%) of the mice immunized with vaccine D-5 were protected whereas 17 of 27 (62.9%) of the mice immunized with vaccine D-6 and 24 of 28 (85.7%)

L-5 = Embryos living 5 days after inoculation.

t Young albino Swiss mice (Webster strain) weighing 12 13 g were used throughout these experiments.

TABLE II.

Comparison of Complement-Fixing Activity, Rickettsial Content and Antigenicity of Vaccines Prepared from Embryos Harvested Before and at the Peak of Embryo Deaths.

Trom 2mory of		Vaccines pr	epared	from y	olk sacs	
		harvest				
		<b>D-5</b>	D-6	D-7	L-7	
No. of yolk saes included in vac	gine	90	102	146	65	
Rickettsial content		± ,	+	++	++++	
Complement fixation test	Vaccine diluted: 1-8	± 3+				
*	1-16	1+	4+	4+	++	
	1-32	1+ ± 0	3+	4+ 3+	4+ 4+ 4+ 1	
	1-64		2+	3+	4+	
	1-128	0	0	0	1	
Antigenicity for mice Method A-0.2 ml undiluted inoculated I.P.	raccine					
Vaccinated group						
Total No. of	mice	28	27		28	
No. protected	Į.	6/28*	17/27	n.t.	24/28	
% protected		21.4	62.9		85.7	
Control group Ch	allenge dose diluted:1-2					0/16*
9 5	1-4					10/15
Method B-0.5 ml of serial vaccine inoculated I.P.	dilutions of					
Vaccinated group	Vaccine diluted: 1-5	0/9	4/9	n.t.	7/10	
•	1-25	0/10	0/10		6/10	
	1-125	0/10	0/10		0/10	
Control group Ch	allenge dose diluted:1-2	•				0/10
<u> </u>	1-4					8/10

D-5 Dead 5 days after inoculation.

of the mice immunized with vaccine L-7 were protected. When mice were immunized by Method B vaccine D-5 diluted 1-5 did not protect any of the 9 vaccinated mice whereas vaccine D-6 diluted 1-5 protected 4 of 9 vaccinated mice, and vaccine L-7 diluted 1-25 protected 6 of 10 vaccinated mice. In addition to the data presented, in 7 additional experiments the complement-fixing activity of the vaccine likewise followed the antigenicity of the vaccine.

Discussion. It is clear from the data presented that complement-fixing activity followed not only the rickettsial content and antigenicity for mice of vaccines prepared in an identical manner but also the toxicity of yolk sac suspensions when care was taken to preserve toxicity. However, the factors responsible for toxicity and complement-fixing activity are not identical since toxicity is destroyed by formalin and ether⁶ while complement-fixing activity is not. Furthermore,

the complement-fixing activity of a given yolk sac suspension or vaccine represents not only the rickettsiæ themselves but also a soluble antigen capable of immunizing guinea pigs. ¹⁰ Nevertheless, when yolk sac suspensions or vaccines were prepared as described from embryos harvested on consecutive days an increase in complement-fixing activity was always accompanied by an increase in toxicity or antigenicity.

Summary. Under the conditions described, the toxicity of untreated yolk suspensions and the antigenicity of typhus vaccines prepared from embryos harvested on consecutive days rose together with the complement-fixing activity of the same antigens.

The authors wish to acknowledge the technical assistance of Mr. Jack O. Davis.

L-7 Living 7 days after inoculation.

⁺ Five rickettsiae per field.

⁺⁺⁺⁺ Over 100 rickettsiae per field.

⁴⁺ Complete fixation of complement.

⁰ No fixation of complement.

^{*} No. of mice surviving/Total No. of mice.

I.P. Intraperitoneally.

¹⁰ Topping, N. H., and Shear, M. J., Pub. Health Rep., 1944, 59, 1671.

		Suspensie	ms.					
Interval between inoculation and harvest	No. of yolk sacs represented	susper	nent fix usion di 1–32		sus	ensi	titra on dula 1–8	
L-5 L-6 L-7 L-8	13 13 13	0 3+ 4+ 4+	0 1+ 2+ 4+	0 ± ± 2+	0/3† 4/4	4/4	$0/4 \\ 1/4$	

TABLE I.

Comparison of Toxicity with the Complement-Fixing Activity of Infected Yolk Sac
Suspensions.*

the tail vein of mice.† The number of deaths was recorded 18 hours after inoculation.

The approximate rickettsial content of a vaccine was estimated by microscopic examination of films of vaccine made with a standard loop and stained by Macchiavello's technic  $(c.f.^{7})$ .

Antigenicity was measured by immunizing mice as follows: Method A: Each of 30 mice was given one intraperitoneal inoculation of 0.2 ml of undiluted vaccine. Method B: Groups of 10 mice each were given one intraperitoneal inoculation of 0.5 ml of vaccine diluted 1-5, 1-25, and 1-125 respectively. Fourteen days after vaccination all vaccinated mice were challenged with 0.5 ml of toxic substance representing 2-4 LD₅₀ injected into the tail vein. The results were recorded 18 hours after challenge.

Experimental. Comparison of toxicity with complement-fixing activity of injected yolk sac suspensious. Inasmuch as both the rickettsial content and complement-fixing activity of vaccines were highest when the constituent yolk sacs were harvested from embryos still living at the peak of embryo deaths,7 yolk sacs were harvested only from living embryos to prevent loss of toxicity following death of the embryo. In comparing toxicity with complement-fixing activity yolk sacs were harvested from 15 living embryos on the 5th, 6th, 7th, and 8th day after inoculation respectively. Each pool of yolk sacs thus obtained was frozen immediately and stored at -70°C. Approximately one week later 10% untreated tissue suspen-

sions in saline were prepared from each pool of yolk sacs and each suspension was titrated for complement-fixing activity and for toxicity in mice. It will be seen from Table I that the complement-fixing titer of yolk sac suspensions increased from less than 1-16 in the case of suspension L-5, prepared from embryos harvested 5 days after inoculation, to 1-32 in the case of suspension L-8, prepared from embryos harvested 8 days after inoculation. When the same suspensions were titrated for the presence of toxic substance by intravenous injection of mice suspension L-5 diluted 1-2 was not lethal for mice whereas suspension L-8 diluted 1-8 was lethal for all mice inoculated. It is thus evident that the complement-fixing activity and toxicity of untreated saline suspensions of infected yolk sacs harvested from living embryos rose together.

Comparison of antigenicity with complement-fixing activity and rickettsial content of The complement-fixing typhus vaccines. activity, rickettsial content and antigenicity of typhus vaccines prepared from yolk sacs harvested before and at the peak of embryo deaths were compared and the results are presented in Table II. It was found that the complement-fixing titer increased from less than 1-8 in the case of vaccine D-5, prepared from embryos dead 5 days after inoculation, to 1-64 in the case of vaccine L-7, prepared from embryos still living 7 days after inoculation and that the rickettsial content of the vaccines increased with the complement-fixing activity. When mice were immunized by Method A only 6 of 28 (21.4%) of the mice immunized with vaccine D-5 were protected whereas 17 of 27 (62.9%) of the mice immunized with vaccine D-6 and 24 of 28 (85.7%)

^{* 10%} tissue suspensions by weight.

t No. of mice dead/ Total No. of mice.

L-5 = Embryos living 5 days after inoculation.

[†]Young albino Swiss mice (Webster strain) weighing 12-13 g were used throughout these experiments.

TARLE II.

Comparison of Complement Fixing Activity, Rickettsial Content and Antigementy of Vaccines Prepared from Embryos Harvested Before and at the Peak of Embryo Deaths.

		Vaccines pr	ed fron	ı embry	yos:	
		D 5	D 6	D 7	L 7	
No. of yolk sacs included in va- Rickettsial content		90 ± 3+ 1+ ± 0 0	102 +	146 ++	65 ++++	
Complement fixation test	Vaccine dilnted: 18	3+				
	1 16	1+	4+	4+	4+	
	1 32	± .	3+	4+ 4+ 3+	4+ 4+ 4+ 1	
	1 64	0	2+	3+ 0	4-	
	nice d d hallenge dose dilnted:12 14	28	0 27 17/27 62 9	Ü	28	0/16* 10/15,
Method B—0.5 ml of serial vaccine inoculated I.P. Vaccinated group  Control group C		0/9 0/10 0/10	4/9 0/10 0/10	n.t.	7/10 6/10 0/10	0/10 8/10

D 5 Dead 5 days after moculation.

of the mice immunized with vaccine L-7 were When mice were immunized by Method B vaccine D-5 diluted 1-5 did not protect any of the 9 vaccinated mice whereas vaccine D-6 diluted 1-5 protected 4 of 9 vaccinated mice, and vaccine L-7 diluted 1-25 protected 6 of 10 vaccinated mice. In addition to the data presented, in 7 additional experiments the complement-fixing activity of the vaccine likewise followed the antigenicity of the vaccine

Discussion. It is clear from the data presented that complement-fixing activity followed not only the rickettsial content and antigenicity for mice of vaccines prepared in an identical manner but also the toxicity of yolk sac suspensions when care was taken to preserve toxicity. However, the factors responsible for toxicity and complement-fixing activity are not identical since toxicity is destroyed by formalin and ether6 while complement-fixing activity is not. Furthermore, 4+ Complete fixation of complement.

0 No fixation of complement.

* No. of mice surviving/Total No. of mice.

I.P. Intraperitoneally.

the complement-fixing activity of a given yolk sac suspension or vaccine represents not only the rickettsiæ themselves but also a soluble antigen capable of immunizing guinea pigs.10 Nevertheless, when yolk sac suspensions or vaccines were prepared as described from embryos harvested on consecutive days an increase in complement-fixing activity was always accompanied by an increase in toxicity or antigenicity.

Summary. Under the conditions described, the toxicity of untreated yolk suspensions and the antigenicity of typhus vaccines prepared from embryos harvested on consecutive days rose together with the complement-fixing activity of the same antigens.

The authors wish to acknowledge the technical assistance of Mr. Jack O. Davis.

L-7 Living 7 days after inoculation.

⁺ Five rickettsiae per field. ++++ Over 100 rickettsine per field.

n.t. Not tested.

¹⁰ Topping, N. H., and Shear, M. J., Pub. Health Rep, 1944, 59, 1671.

#### 15186

### Synergistic Action of Nicotinamide Upon Penicillin.

EDWARD H. FRIEDEN. (Introduced by C. N. Frazier.)

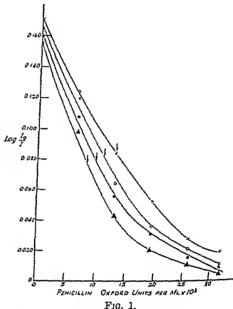
From the Department of Dermatology and Syphilology, The University of Texas School of Medicine, Galveston, Texas.

In the course of some studies dealing with the mechanism of penicillin action, it was observed that relatively high concentrations of nicotinamide appeared to increase measurably the effectiveness of penicillin against the Oxford strain of Staphylococcus aureus. Since, of the B vitamins, only para-aminobenzoic acid has been shown to affect penicillin activity, 1,2 this synergistic action has been investigated in more detail.

Experimental Methods. In testing the effectiveness of compounds under investigation for their synergistic action, it was found most convenient to compare the concentrations of penicillin necessary to produce half-maximum inhibition of the test organisms in the presence and absence of the agent under study. The organisms being investigated were grown in a modified proteose-peptone medium, with added glucose and yeast extract. Short (5-7 hour) growth periods were used. Growth levels were estimated turbidimetrically, using a Klett-Summerson photoelectric colorimeter.

In most of the experiments, Lederle penicillin (potency 400 Oxford units per mg) was used. Crystalline sodium penicillin G was obtained through the courtesy of Merck and Co. Eastman Kodak Company nicotinamide and nicotinic acid were used without purification, except as noted below.

Results. The addition of nicotinamide to the culture medium in concentrations less than about 250  $\gamma$  per ml has no apparent effect upon the inhibition of Staphylococcus aureus by penicillin. Beyond this point, however, significant effects are observed. Representative data are shown in Fig. 1. It is evident that the concentration of penicillin necessary for half-maximum inhibition decreases with increasing concentration of the amide. Con-



Effect of increasing concentrations of nicotinamide upon penicillin inhibition of Staphylococcus aureus (Oxford). The concentrations of nicotinamide, in mg/ml, are as follows: X—0.00; 0—0.40; 0—0.80; 1—1.60. The vertical arrows indicate half-maximum inhibition points.

centrations of nicotinamide greater than 1.7 mg/ml were not studied.

Despite the fact that nicotinic acid is frequently interchangeable with nicotinamide in bacterial nutrition,³ it was found that sodium nicotinate was unable to duplicate the action of nicotinamide when used in comparable concentrations.

The relatively high nicotinamide levels necessary to produce observable synergism suggested the possibility that the effects noted were in reality due to an impurity in the preparation used, although two different lots of nicotinamide, obtained from the same manufacturer at different times, gave identical

¹ Ungar, J., Nature, 1943, 152, 245.

² Hobby, G. L., and Dawson, M. H., Proc. Soc. Exp. Biol. And Med., 1944. 56, 184.

³ Peterson, W. H., and Peterson, M. S., Bact. Rev., 1945, 9, 49.

results. That this was not the case was shown by the fact that purified nicotinamide, recrystallized from either dioxane or alcoholic benzene, gave results which were indistinguishable from those obtained with the cruder preparation. Likewise, identical results were had, when the impure penicillin preparations were replaced by crystalline penicillin at comparable concentrations.

The possibility that the effects observed were due to a slow reaction of penicillin with nicotinamide to form a product superior in antibacterial activity to penicillin itself was next investigated. On the basis of this hypothesis, the high nicotinamide concentrations required are necessary to force the reaction measurably toward completion in the relatively short periods involved. Accordingly, mixtures of nicotinamide and penicillin (including mole ratios from 4:1 to 4,000:1) were incubated at 37°. After 18 hours, the solutions were diluted so that the residual nicotinamide concentrations were less than 20 y per ml, and inhibition curves determined. These were compared with curves for a solution of penicillin alone which had been treated similarly. No enhancement of penicillin action could be observed under these conditions.

In an effort to determine the effect of nicotinamide upon penicillin inhibition of other organisms, experiments similar to those described above were performed using Streptococcus pyogenes C-203, E. coli, and two other strains of Staphylococcus aureus. Penicillin inhibition of S. pyogenes and E. coli was unaffected by the presence of even large amounts of nicotinamide. However, the other strains of Staphylococcus aureus studied responded to combinations of penicillin and nicotinamide

in a manner qualitatively and quantitatively analogous to the response of the Oxford staphylococcus. In the presence of 1.5 mg of nicotinamide per ml, the concentration of penicillin required for half-maximum inhibition of the latter organism is approximately 0.7 that required when no nicotinamide is present. Ratios for the other strains studied were 0.64 and 0.67 respectively. It should be noted that these 3 strains had significantly different penicillin sensitivities.

It is well known that penicillin is most effective under conditions favoring rapid growth of the organism, and the theory that penicillin acts primarily upon actively multiplying cells is supported by a good deal of evidence. It seems unlikely, however, that the phenomenon under discussion can be explained by an increased rate of cellular multiplication. Although detailed studies of the effect of nicotinamide upon the rate of growth of the staphylococcus have not been made, a single experiment indicated that a slight decrease in rate occurred upon using concentrations of nicotinamide comparable to those indicated above.

Summary. High concentrations of nicotinamide exert a synergistic effect upon penicillin inhibition of Staphylococcus aureus. Nicotinic acid was inactive in this respect. No evidence for a reaction between nicotinamide and penicillin could be found. Although the effect of nicotinamide appeared to be general for the 3 strains of staphylococcus tested, no effect could be demonstrated upon either Streptococcus pyogenes or E. coli.

⁴ Chain, E., and Duthie, E. S., Lancet, 1945, 1, 652.

#### 15187 P

## Studies on the Action of Penicillin. IV. Development of Penicillin Resistance by Gonococcus.*

C. PHILLIP MILLER AND MARJORIE BOHNHOFF.

From the Department of Medicine and the A. B. Kuppenheimer Foundation, University of Chicago.

Among the several recent reports on the development of penicillin resistance by various microörganisms, only that by Bahn, Ackerman, and Carpenter¹ concerns gonococcus. They were able to enhance the penicillin tolerance of one of 5 strains of gonococcus sufficiently after 32 weeks to permit growth in blood broth containing 2 units of penicillin per ml. The other strains responded less readily, one scarcely at all. Lankford² found that among 203 freshly isolated strains of gonococcus none was resistant to more than 0.02 units of penicillin per ml.

As gonococci grow more readily on solid media than in broth, the following experiments were made by surface cultivation on agar containing known concentrations of penicillin.

Methods. Strains of gonococci were transferred daily to freshly prepared blood agar plates³ containing increasing concentrations of penicillin.[†] Each transfer was made to 3 or 4 plates containing graded increments of penicillin and taken from the plate which had

developed confluent growth during 24 hours incubation. This procedure was followed because it was found that enhancement of penicillin tolerance proceeded more rapidly when transfer was made from media containing the highest concentration of penicillin which permitted moderately abundant growth rather than from higher concentrations which produced only a few colonies.

The penicillin agar was prepared each day just before use because its growth inhibiting potency was found to diminish even in the ice-box.

The results with one strain are plotted in Fig. 1. The penicillin tolerance; that is, the ability to multiply on penicillin agar, increased rapidly at first and reached its limit at 7.7 units per ml. Just before that occurred and at a time when the average daily increment had become small, a second series of transfers was begun by subcultivation onto penicillinfree agar. Thereafter, this second series was transferred back and forth from penicillincontaining to penicillin-free agar. As will be seen in the chart, this practice resulted in further and more rapid enhancement of penicillin tolerance which reached a level of 21 units per ml. The only detectable difference in this second series of transfers was more luxuriant growth on penicillin-free agar which provided a greater number of viable gonococci to be carried back onto penicillin agar. These results indicate that enhancement of penicillin resistance proceeds most rapidly when vigorous multiplication is maintained.

To test the validity of the foregoing explanation, a strain of gonococcus was repeatedly subjected to prolonged exposure to concentrations of penicillin which inhibited reproduction. To 2 cc volumes of blood broth containing varying concentrations of penicillin, approximately  $5 \times 10^6$  gonococci per ml were

^{*}A portion of the work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

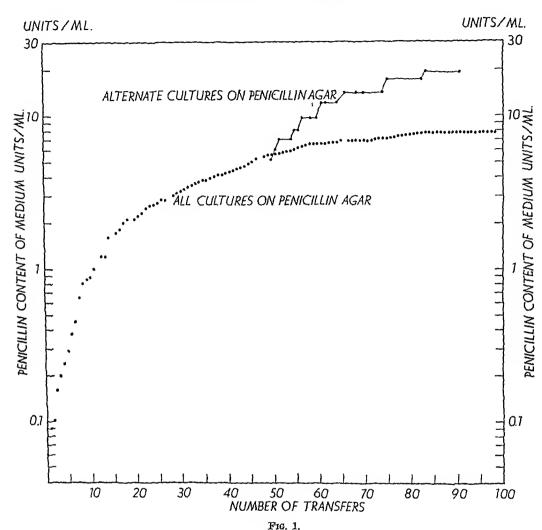
¹ Bahn, Jeanne M., Ackerman, Helen, and Carpenter, Charles M., Proc. Soc. Exp. Biol. And Med., 1945, 58, 21.

² Lankford, Charles E., Am. J. Syph. Gon. and Ven. Dis., 1945, 29, 56.

³ The medium is described by Miller, C. Phillip, Moeller, Velma, and Bohnhoff, Marjoric, Proc. Soc. Exp. Biol. AND Med., 1945, 58, 143.

t The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

## ABILITY OF GONOCOCCUS TO GROW ON MEDIUM CONTAINING INCREASING CONCENTRATIONS OF PENICILLIN



mixed in Wassermann tubes, incubated overnight, then centrifuged at high speed for one-half hour. The number of viable gonococci surviving was determined by culturing the centrifuged sediment onto freshly poured blood agar plates which were incubated in a candle jar and examined at 24 and 48 hours. After the first 24 hours of incubation, colonies developing from organisms surviving the highest concentration of penicillin were grown on penicillin-free agar and used for the next suspension in penicillin broth.

After 13 successive suspensions in penicillinbroth, the number of viable gonococci surviving exposure to each concentration of penicillin had not increased (beyond the limits of the error of the method) and indicate that the resistance of the strain had not been appreciably increased.

At the time of the first penicillin broth suspension and again after the 9th, the strain was tested for its ability to multiply on penicillin agar. The change was insignificant, being 0.04 and 0.05 units per ml, respectively.

The reliability of the method was checked by subjecting to this treatment organisms which had developed ability to multiply on agar containing different concentrations of penicillin. Multiplication always occurred in broth containing penicillin in the concentration just below that of the agar from which the inocula were taken.

Penicillin-fast strains of gonococcus showed distinct morphological changes, which were retained during subsequent cultivation on penicillin-free agar. They will be described in a subsequent report.

Penicillin resistance developed in vitro was

not permanently retained during prolonged cultivation on penicillin-free agar. Its rate of loss has not yet been accurately determined.

Summary. Penicillin-fastness developed most rapidly in gonococci under conditions which permitted the greatest number of viable microörganisms to be transferred to a higher concentration of penicillin at each transfer. One strain of gonococcus acquired the ability to grow on media containing 21 units per ml. No appreciable increase in penicillin tolerance resulted from repeated exposure to bacteriostatic concentrations of penicillin.

#### 15188 P

Studies on Action of Penicillin. V. Virulence of Penicillin Resistant Strains of Meningococcus.

C. PHILLIP MILLER AND MARJORIE BOHNHOFF.

From the Department of Medicine and the A. B. Kuppenheimer Foundation, University of Chicago.

Several reports have appeared on the development of penicillin resistance in vitro and in vivo by staphylococci1,2 and pneumococci,3 but they do not agree on its effect on virulence.

Methods. The sensitivity of meningococci to the bacteriostatic action of penicillin* was determined by transferring them from routine media onto a series of blood agar plates4 containing known concentrations of penicillin and incubating them in a candle jar. The highest concentration permitting the development of any colonies was considered the limit of that strain's resistance to penicillin. Increased resistance to penicillin was developed by the method described for gonococci.5 was determined by inoculating mice intraperitoneally with mucin suspensions of meningococci.6 Susceptibility to the therapeutic action of penicillin was estimated by infecting mice with 10,000-100,000 M.L.D. of meningococci and injecting them subcutaneously 3 and 6 hours thereafter with appropriate doses of penicillin.

The natural resistance to peni-Results. cillin was determined on 96 strains of meningo-

cocci and found to vary from 0.1 to 0.5 units per ml. Seven strains were selected at random for enhancement of penicillin resistance and were subcultured daily on media containing increasing concentrations of penicillin. The resistance of all 7 strains increased at about

1 McKee, Clara M., and Houck, Carol L., PROC. Soc. EXP. BIOL. AND MED., 1943, 53, 33.

² Spink, Wesley W., Hall, Wendell H., and Ferris, Viola, J. A. M. A., 1945, 128, 555.

3 Sehmidt, L. H., and Sesler, Clara L., Proc. Soc. EXP. BIOL. AND MED., 1943, 52, 353.

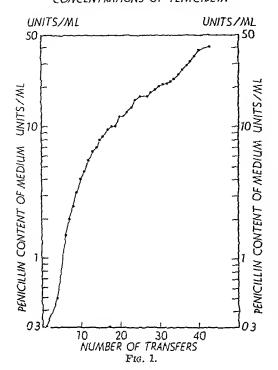
* The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigations recommended by the Committee on Chemotherapeutic and Other Agents of the National Research Council.

4 The medium is described by Miller, C. Phillip, Moeller, Velma, and Bohnhoff, Marjorie, Proc. Soc. Exp. Biol. and Med., 1945, 58, 143.

5 Miller, C. Phillip, and Bohnhoff, Marjorie, PROC. Soc. EXP. BIOL. AND MED. (preceding comnunication).

6 Miller, C. Phillip, and Castles, Ruth, J. Inf. Dis., 1936, 58, 263.

ABILITY OF MENINGOCOCCUS TO GROW ON MEDIUM CONTAINING INCREASING CONCENTRATIONS OF PENICILLIN



the same rate. None was carried to its limit. The one strain (No. 274) which was carried farthest became contaminated at a time when it was growing on media containing 41 units per ml. The results with this strain are shown in Fig. 1.

The virulence of all 7 strains was determined after they had acquired varying degrees of penicillin resistance up to 5 units per ml and all were found to have lost very little or none of their original virulence for mice.

Table I summarizes observations on the in vitro and in vivo action of penicillin on strain 274 up to the level at which it lost virulence. These include determinations of virulence and susceptibility to therapeutic action in the infected mouse. The slight diminution of virulence which occurred as penicillin resistance developed was no more

TABLE I.
Changes in Virulence and in Susceptibility to
Treatment by Penicillin During the Development
of Penicillin Resistance by Meningoeoccus.

Virulence*	Smallest protective dose of penicillin† units
maximal	20-40
high	400
ĭ1	600
"	600
none	not done
	maximal high

* maximal—M.L.D. of 1-10 meningococci high—M.L.D. of 10-100 meningococci, none—M.L.D. of 10⁹ meningococci.

f Smallest dose of penicillin which successfully protected mice infected with 100,000 of the meningococci described in the other columns.

than commonly occurs during repeated subcultivation on artificial media. But when the strain had acquired resistance to 18 units per ml virulence was lost completely and could not be restored by mouse passage. The strain had also acquired unusual physical properties although it still retained its ability to ferment glucose and maltose.

The table also shows that as the strain increased its tolerance in vitro, the test infection (always produced by inoculating the same number of microörganisms) required larger doses of penicillin for successful treatment. Mice infected with the strain when it was resistant to 14 units per ml required 600 units of penicillin for its control, a dose 15-30 times the number which was effective before the strain had begun to acquire penicillin-resistance.

Summary. None of 96 strains of meningo-cocci was found to be naturally resistant to penicillin in vitro. Seven strains all acquired penicillin resistance readily and at about the same rate during repeated subcultivation on agar containing increasing concentrations of penicillin. The strains remained virulent for mice as penicillin resistance increased, except 1 strain which lost virulence at 18 units per ml. Infections produced with penicillin-resistant strains required very large doses of penicillin for their control.

#### 15189

## Antibody Response of Swine to Vaccination with Formolized Swine Influenza Virus Adsorbed on Alum.*

I. W. McLean, Jr., Dorothy Beard, A. R. Taylor, D. G. Sharp, and J. W. Beard, From the Department of Surgery, Duke University School of Medicine, Durham, N.C.

The antibody response of swine to vaccination with formolized influenza virus suspended in saline solution is characterized in part^{1,2,3} both by a swift increase in antibody titer and by a subsequent rapid decline in the titer to low levels. A similar transient effect of influenza virus vaccines has been observed in man. Because of this rapid loss of circulating antibody, various substances have been combined with the inactivated virus to alter the adsorptive and immunological processes for the purpose of prolonging the period of high antibody titer. Friedewald^{5,6} reported

*This work was supported through the Commission on Influenza and the Commission on Epidemiological Survey, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army. The work was also aided in part by a grant to Duke University from Lederle Laboratories, Inc., Pearl River, New York.

t Member, Commission on Epidemiological Survey, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U. S. Army.

Consultant to Secretary of War and Member, Commission on Acute Respiratory Diseases, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, U.S. Army.

¹ McLean. I. W., Jr., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., Science, 1945, 101, 544.

² McLean, I. W., Jr., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., J. Immunol., 1945, 51, 65.

3 McLean, I. W., Jr., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., Proc. Soc. Exp. BIOL. AND MED., 1945, 60, 152.

4 Hirst, G. K., Rickard, E. R., Whitman, L., and Horsfall, F. L., Jr., J. Exp. Med., 1942, 75, 495.

Friedewald, W. F., Science, 1944, 99, 453.
Friedewald, W. F., J. Exp. Mcd., 1944, 80, 477.

excellent results in mice, rabbits, and ferrets by the use of killed acid-fast bacilli, paraffin oil, and "Falba" with formalin-inactivated influenza virus A (PR8 strain), a mixture which caused much local reaction. Salki found that influenza vaccine adsorbed on calcium phosphate produced better and longerlasting protection in mice than vaccine in saline solution. In rabbits and guinea pigs sterile abscesses frequently occur⁸ at the site of injection of formolized influenza virus precipitated with calcium phosphate.9 Bodily, Corey and Eaton 10 employed alum for concentrating the influenza virus, and in limited tests in man observed no essential difference between the antibody response to alum-adsorbed vaccine and that to the vaccine prepared11 with salt solution. In view of the considerable advantages in the use of alum12,13 with diphtheria and tetanus toxins, the question has been investigated further in quantitative studies on the antibody response of swine to known amounts of formalin-inactivated swine influenza virus adsorbed on alum. sults obtained are reported in the present paper.

Materials and Methods. The stock vaccine, prepared on 5/15/44 and stored at 2-8°C, was identical with that used in a previous study³ and contained 2.0 mg of virus per ml, 0.05% formalin and 1/50,000 phenyl mer-

⁷ Salk, J. E., Science, 1945, 101, 122.

⁸ McLean, I. W., Jr., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., unpublished data.
9 Salk, J. E., Proc. Soc. Exp. Biol. and Med., 1943, 52, 165.

¹⁰ Bodily, H. L., Corey, M., and Eaton, M. D., PROC. Soc. EXP. BIOL. AND MED., 1943, 52, 165.

¹¹ Hirst, G. K., Rickard, E. R., and Whitman, L., PROC. SOC. EXP. BIOL. AND MED., 1942, 50, 129.

¹² Glenny, A. T., and Südmersen, H. J., J. Hyg., 1921, 20, 176.

¹³ Glenny, A. T., Buttle, G. A. H., and Stevens, M. F., J. Path. and Bact., 1931, 34, 267.

	October				November					December				January				
Animal group	16	17	23	24	30	31	6	7	13	20	21	28	4	5	11	18	$\overline{2}$	15
1		BV	*	В		В		В			В			$\bar{\mathrm{B}}$				
2	BV		$\mathbf{B}$		Вľ	•	В		$\mathbf{B}$	$\mathbf{B}$			$\mathbf{B}$			$\mathbf{B}$		
3	BV		$\mathbf{B}$		В		В		BI	т В		В	$\mathbf{B}$			$\mathbf{B}$	В	
4												BV		В	$\mathbf{B}$	$\mathbf{B}$	В	$\mathbf{B}$
5												BV		В	В	В	В	В

TABLE I
Dates of Vaccination and Bleeding.

curic borate. Three separate batches of vaccine, A, B, and C, were prepared using different amounts of alum.

Batch A was prepared on 10/6/44. To 25 ml of the stock vaccine, diluted to 100 ml with sterile Ringer solution and warmed to 30°C, 50 ml of sterile 2.2% alum solution were added to give the concentration of alum commonly employed for preparation of commercial alum-precipitated diphtheria toxoid. The pH was adjusted to 6.5, and a heavy, white, flocculent precipitate formed immediately. After storage of the suspension overnight at 4°C, the floccules were sedimented by centrifugation (500 times gravity), and the clear, supernatant fluid was decanted. sediment was resuspended in 100 ml of sterile Ringer solution containing 0.05% formalin and 1/50,000 phenyl mercuric borate. Hemagglutinative tests showed a loss of less than 1% of the virus, the content of which in the preparation, from nitrogen estimated by Kjeldahl, was 0.56 mg per ml. Vaccine A thus consisted of 0.5 mg (based on the amount of stock vaccine used) of formolized swine influenza virus per ml adsorbed on 1.1% alum and suspended in Ringer-formalin-phenyl mercuric borate solution.

B and C were prepared on 11/27/44 by precipitation on 0.6 and 0.3% alum, respectively. In the instance of B, 12.5 ml of 1.1% alum in formalin-phenyl mercuric borate-Ringer solution were added to 6.25 ml of the original stock vaccine. The pH was adjusted to 6.5-7.0 with 0.2 N NaOH; the resulting precipitate was considerably less dense than

that observed in the preparation of A. Because of possible incomplete adsorption and consequent loss of virus, this precipitate was not sedimented. Instead, Ringer-formalinphenyl mercuric borate solution was added to yield 25 ml of vaccine. For the preparation of Vaccine C, 12.5 ml of a 0.6% alum solution were used in the same manner. Kjeldahl analysis revealed the virus content of B and of C to be 0.54 and 0.53 mg per ml, respectively.

The experimental animals were swine like those previously used.2,3 The various groups for a given experiment were chosen so that each group was comparable with the others with respect to weight and sex. The animals of the first experiment (Groups 1, 2, and 3) were somewhat larger than those usually employed,2.3 ranging in age from 18 to 24 weeks and in weight from 61 to 138 lb, with an average of 82 lb. There were 32 females and 16 males; all of the males except 3 had been castrated. The animals for the second experiment (Groups 4 and 5) were from 16 to 20 weeks old and varied in weight from 48 to 92 lb, with an average of 67 lb. There were 20 females and 12 males; 9 of the males were castrated. A few animals of Poland-China and Duroc strains were used, but the Berkshire strain was predominant.

The animals kept in the usual way,^{2,3} remained healthy except during 2 weeks of abnormally bad weather at the end of December, 1944, when many suffered from upper respiratory infections. One animal of Group 1, 3 of Group 4, and 1 of Group 5 died at this time, of what appeared to be a bacterial pneumonia. There was no evidence of influenza.

Vaccination was performed by introducing 1 ml of the vaccine subcutaneously into the

^{*} B-Bled; V-Vaccinated.

[§] Baker and Adamson Reagent, Crystalline Aluminum Potassium Sulfate,  $Al_2(SO_4)_3K_2SO_4$ . 24H₂O. The solution used was 5% alum on the basis of the hydrated salt.

#### 15189

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³ McLean, I. W., Jr., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., Proc. Soc. Exp. Biol. and Med., 1945, 60, 152.

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5 Friedewald, W. F., Science, 1944, 99, 453.

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Materials and Methods. The stock vaccine, prepared on 5/15/44 and stored at 2-8°C, was identical with that used in a previous study³ and contained 2.0 mg of virus per ml, 0.05% formalin and 1/50,000 phenyl mer-

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⁸ McLean, I. W., Jr., Beard, D., Taylor, A. R., Sharp, D. G., and Beard, J. W., unpublished data-9 Salk, J. E., Proc. Soc. Exp. Biol. AND Med., 1943, 52, 165.

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¹³ Glenny, A. T., Buttle, G. A. H., and Stevens, M. F., J. Path. and Bact., 1931, 34, 267.

gain in weight was 8 lb per animal per week.

Bleeding, preparation of sera, storage, and titration of the capacity of the sera to inhibit the hemagglutinative reaction were carried out as previously described.^{2,3} Sera obtained immediately before, and 1, 2, 3, 5, and 7 weeks after, vaccination were titrated twice; (1) without freezing and within 4 days of the time of bleeding, and (2) after storage in the frozen state until the completion of both experiments. The titers of the 2 sets of titrations exhibited a standard deviation of 0.47 of a 2-fold dilution, and the values given here were the geometric means of the two endpoints.

Use was made of the geometric means of the titers of the individual pigs for the reasons previously given.²

Results. The experiments were set up in two categories. The purpose of the initial experiment was to measure the antibody response to the vaccine adsorbed on alum and to test the response to a second vaccination with the same material after intervals of 2 and 4 weeks. Three groups of 16 pigs each were given a single dose of Vaccine A (1.1% alum), and Groups 2 and 3 were revaccinated after intervals of 2 and 4 weeks, respectively. The sequence of vaccination and bleeding is shown in Table I.

In the second experiment the relation of the response to the quantity of alum employed for adsorption was investigated. Two groups of animals, 5 and 6, were used; Group 5 was given a single dose of Vaccine B (0.6% alum) and Group 6 a single dose of Vaccine C (0.3% alum). The time relations of vaccination and bleeding in this experiment are likewise shown in Table I.

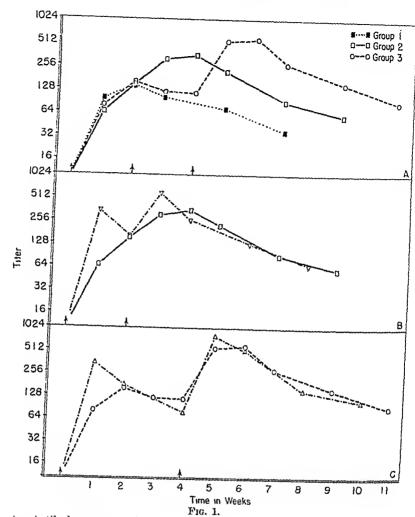
The results of experiment 1, Groups 1, 2, and 3, are shown in Fig. 1, A. The antibody titer rose sharply during the first week after the initial injection and reached the maximum at the 2-weeks interval. The rate of antibody loss in subsequent weeks is illustrated in the findings with Group 1. Repetition of vaccination of Group 2 at the 2-weeks interval resulted in a continuation of the rise initiated by the first injection to a much higher level within 1 week and maintenance of this level for another 7 days. In the instance of Group 3, a considerable diminution in antibody

occurred in the interval between first and second vaccinations; nevertheless, the second vaccination of this group resulted in a rapid increase in titer, which reached a level in 1 week still higher than that of the group revaccinated after the 2-weeks interval. The high level was maintained for a period of 7 days. The rate of antibody loss after final vaccination was nearly the same in all of the groups.

In Fig. 1, B and C, are shown comparisons of the responses to repeated vaccination at the intervals of 2 (Fig. 1, B) and 4 (Fig. 1, C) weeks with vaccine adsorbed on alum (Groups 2 and 3, Fig. 1, A), and with the same vaccine without alum (Fig. 2, Groups B and D).3 The results shown in Fig. 1, B and C, show that the overall responses to the two sorts of vaccine were strikingly similar both in degree and duration. Differences were seen only in the rate of response and in the maximum immediate response. The highest titer after the initial vaccination with alum vaccine occurred at the 2-weeks interval; response was more rapid to the second vaccination, a high titer being reached in 7 days and maintained for another week before loss occurred. The rates of loss of antibody titer afterward were essentially identical in both instances.

The effects of one injection of vaccine adsorbed on various quantities of alum are shown in Fig. 2 (Experiment 2, Groups 4 and 5). The findings of Group 1 (Fig. 1, A) which received a single injection are repeated in Fig. 2 to compare more clearly the effects of 1.1% alum. It is seen again that the rate of response is relatively slow and, with all quantities of alum employed, the maximum titer was reached 2 weeks after vaccination. It is evident that the degree of response was considerably affected by the amount of alum employed. The highest mean titer observed with the vaccine in the presence of 0.3% alum was 277 and that with vaccine adsorbed on 1.1% alum was 147, a 2-fold difference in titer.

Discussion. Swine influenza virus vaccine adsorbed on alum elicited an antibody response in swine which differed in only two evident respects from the response to the identical kind and amount of the vaccine dispersed in saline solution. These differences were (1) a brief delay, approximately 1 week, in the



A. Antibody response of swine to vaccination with 0.5 mg of formolized swine influenza virus adsorbed on 1.1% alum. Group 1 received but one vaccination; in Groups 2 and 3 vaccination was repeated after 2 and 4 weeks, respectively.

B. Comparison of response to repeated vaccination after 2 weeks with alumprecipitated vaccine (Group 2, Fig. 1, A) with response to like vaccination with the same vaccine without alum (Group B, Fig. 2, previously reported³).

C. Comparison of response to repeated vaccination after 4 weeks with alumprecipitated vaccine (Group 3, Fig. 1, A) with response to like vaccination with the same vaccine without alum (Group D, Fig. 2, previously reported.).

right posterior axillary region where fat was avoided. Second injections were given similarly on the left side. No general reactions were observed, but all of the animals developed indurated nodules at the site of vaccination. The nodules were larger and more persistent with the larger amounts of alum. Those associated with the vaccine adsorbed on 1.1% alum were about 1.5 to 2 cm in diameter; about

70% of those initially present were still palpable after 7 weeks. The 0.6 and 0.3% alum vaccines produced nodules averaging 1 and 0.7 cm, respectively, which persisted for 7 weeks in 56 and 38% of the 2 groups of animals.

The pigs were weighed at the time of each vaccination and at the end of the experiment 7 weeks after the last vaccination; the average

#### Inhibition of Salmonella Cultures by Streptomycin.

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Streptomycin is known to be active against various gram-negative bacteria, but little is known of its action on Salmonella. The present paper is a report of the *in vitro* inhibition of 412 Salmonella cultures by the agent. The cultures employed in the work included 154 distinct serological types which comprised all the presently recognized forms in the genus. Only one or two representatives of the majority of the types were studied since most of them occur very rarely. A number of the types which occur more frequently and which are better known were represented by a larger number of cultures.

The tests were performed by streaking tryptose agar plates which contained varying amounts of streptomycin with a 2 mm loopful of a saline suspension which contained approximately 100 million cells per ml. Streptomycin was added in amounts of 1, 2, 4, 8, 16, 32, and 64 units per ml of agar. Two samples of streptomycin which contained respectively 80 and 120 units per mg were used and similar results were obtained with both samples.† Streptomycin was added to the melted and cooled agar which was poured into plates and streaked immediately after hardening with freshly prepared bacterial suspensions. plates were incubated 24 hours at 37°C. The smallest amount of streptomycin which suppressed all visible growth of the bacteria was recorded as the inhibitive concentration.

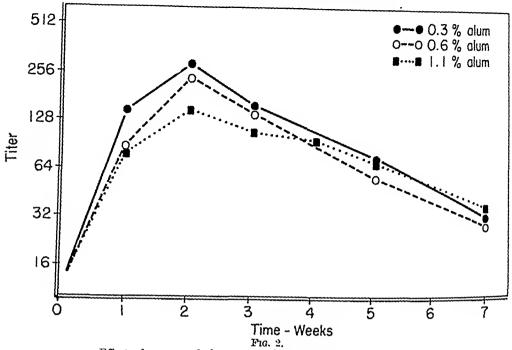
It was not found practical to space the dilutions of streptomycin more closely than the serial dilutions mentioned above since there was a gradual inhibition of growth as the amount of streptomycin increased. In some cultures a few resistant colonies developed on plates which contained the higher concentrations. In others complete inhibition was preceded by the occurrence of pin-point growth in one or two dilutions. Many of the tests were repeated several times and comparable results were obtained. The point of complete inhibition sometimes varied one dilution from test to test, *i.e.*, a given culture might be inhibited by 8 units in one test and by 16 units in a second. In most instances the end-point was the same in repeated trials.

Strain W of E. coli was used to control all tests and this culture was inhibited regularly by 4 units of streptomycin per ml of medium. The Salmonella strains in general were more resistant; the great majority of the types were inhibited by 8 to 16 units per ml. Space does not permit the separate listing of all the types tested but it may be said that with the exception of the types enumerated below and the cultures given in Table I, all the cultures were inhibited either by 8 or 16 units of streptomycin per ml. S. typhi suis, S. duesseldorf, S. sendai and S. poona were inhibited by 2 units of streptomycin per ml, while 4 units were required to inhibit S. abortus-ovis, S. muenchen, S. dublin, S. rostock, S. moscow, S. blegdam, S. selandia, S. hvittingfoss, S. minnesota, S. meleagridis, S. orion, and S. pretoria. Those cultures which were more resistant than the majority of the types required 32 units per ml to inhibit growth, Among this group were S. amager, S. california, S. manhattan, S. altendorf, S. simsbury, S. mississippi, S. tennessee, S. florida, S. madelia, and S. heves.

In Table I are listed 252 cultures of 19 of the types frequently encountered. Different cultures of the same type vary widely in their sensitivity to streptomycin and it is unwise to draw conclusions regarding the susceptibility of a given type unless several cultures are

^{*} The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

t The samples of streptomyein were obtained through the courtesy of Merck and Company and their receipt is gratefully acknowledged.



Effect of amount of alum present in vaccine on antibody response.

attainment of the maximum antibody level and (2) a lower antibody titer at the time of peak response. At no time was the response to the alum vaccine higher than that to the vaccine without alum. It is of interest to note that the maximum antibody titer induced by the vaccine with alum, which came at the interval of 2 weeks after vaccination, was essentially identical with the declining titer following the use of plain vaccine at the same interval. The results in swine were similar in this respect to those in man described by Bodily, Eaton and Corev. 10 for these authors measured antibody content at the interval of 2 weeks after vaccination with the two types of vaccine. Subsequent to the 2-weeks interval, the rates of antibody decline in swine were the same with the 2 vaccines and, consequently, the levels of antibody titer were alike with respect to time.

It was observed, further, that adsorption of the vaccine on alum may lessen the response to a given amount of the vaccine. In the present experiments, there appeared to be a definite relation between the height of response and the quantity of alum used. The response was least in the instance of 1.1% alum and greatest in that of 0.3% of the material. The present results as a whole indicate not only that adsorption of influenza virus vaccine on alum is of no benefit but that it may possibly be detrimental to the production and maintenance of anti-influenza antibody titer.

Summary. The antibody response of swine to formolized swine influenza virus adsorbed on alum is essentially the same as that to the vaccine dispersed in saline solution. The chief influence of the alum is a brief delay in the peak response. The higher concentrations of alum lessen the primary antibody response, but the subsequent antibody levels and the rates of diminution in titers are nearly identical with the two sorts of vaccine.

#### 15191

#### Cytotoxicity of Streptomycin and Streptothricin.

DOROTHY H. HEILMAN. (Introduced by H. C. Hinshaw.)
From the Division of Clinical Pathology, Mayo Clinic, Rochester, Minn.

The present study was done as part of a clinical and laboratory investigation of streptomycin, an antibiotic agent described by Schatz, Bugie, and Waksman.1 Streptomycin has been found effective in the treatment of a number of experimental infections in laboratory animals.2-10 In experiments reported by Feldman and Hinshaw⁷ and by Feldman, Hinshaw, and Mann,10 streptomycin was administered to guinea pigs over a long period without causing recognizable toxic reactions or tissue changes. Of several different lots of streptomycin used in the experiments of Feldman and Hinshaw, only one appeared to be toxic, indicating that the reactions observed were probably due to an impurity and not to streptomycin itself.

The tests of cytotoxicity reported in this paper were carried out on a number of samples of streptomycin before they were used for clinical investigation. In addition, a limited number of tests was also done with streptothricin, an agent described by Waksman¹¹ that is similar in many respects to streptomycin, especially with regard to its selective anti-bacterial activity. Robinson, Graessle, and Smith⁵ have reported on the toxicity of streptothricin for mice. In another study they reported that some samples of streptomycin are several times less toxic than streptothricin in acute toxicity tests.⁶ Feldman and Hinshaw¹² found streptothricin toxic for guinea pigs when it was given by repeated administration.

Experimental Methods. The tissue culture technic used has been described previously in detail.13 Young adult male rabbits were used exclusively as a source of tissue, plasma, and serum used in the preparation of cultures. A serum-chick-embryo extract was made by extracting chick embryos of 8 days of incubation with rabbit's serum in the proportion of 1 embryo to 5 cc of serum. Fragments of rabbit's spleen measuring approximately 1 to 2 mm across were selected and were placed in Tyrode's solution in matched groups of 12 fragments each. In each experiment 1 group of 12 fragments was used in the preparation of the control series of cultures and each of the remaining groups comprised a test series. A solution of streptomycin or streptothricin* in Tyrode's solution was added to the tissue extract used in the preparation of the test cultures in an amount that would comprise 1/20

¹ Schatz, Albert, Bugie, Elizabeth, and Waksman, S. A., Proc. Soc. Exp. Biol. and Med., 1944, 55, 66.

² Jones, Doris, Metzger, H. J., Schatz, Albert, and Waksman, S. A., Science, 1944, n.s. 100, 103,

³ Waksman, S. A., Bngie, Elizabeth, and Schatz, Albert, Proc. Staff Mcct., Mayo Clin., 1944, 19, 537.

⁴ Heilman, F. R., Proc. Staff Meet., Mayo Clin., 1944, 19, 553.

⁵ Robinson, H. J., Graessle, O. E., and Smith, Dorothy G., Science, 1944, n.s. 99, 540.

⁶ Robinson, H. J., Smith, Dorothy G., and Gracssle, O. E., Proc. Soc. Exp. Biol. AND Med., 1944, **57**, 226.

⁷ Feldman, W. H., and Hinshaw, H. C., Proc. Staff Meet., Mayo Clin., 1944, 19, 593.

⁸ Heilman, F. R., Proc. Staff Meet., Mayo Clin., 1945, 20, 33.

⁹ Heilman, F. R., Proc. Staff Meet., Mayo Clin., 1945, 20, 169.

¹⁰ Feldman, W. H., Hinsbaw, H. C., and Mann, F. C., annpublished data.

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¹³ Herrell, W. E., Heilman, Dorothy, and Gage, R. P., Am. J. M. Sc., 1943, 206, 26.

^{*} Samples of streptomycin and streptothricin were kindly furnished by Dr. Waksman and also by Dr. D. F. Robertson of Merck & Co., Inc. Additional samples of streptomycin were furnished through the kindness of Abbott Laboratories, Parke, Davis & Company, and the Upjohn Company.

TABLE I.
Inhibition of Salmonella Cultures by Streptomyon

						nlubited by medium		
Type	No. of cultures tested	1	2	4	8	16	32	
S. paratyphi A	8		1	6	1			
S. paratyphi B	24			2	13	7	_	
S. typhi murium	28			2	13	9	-	
S. derby	12		1	1	8	2		
S. paratyphi C	3				3			
S. cholera vais	12			5	<b>5</b>	1		
S. cholcræ-suis var. Lunzendorf	16			1	7	8		
S. montevideo	12				4	7		
S. oranienburg	13			2	9	2		
S. barcilly '	11				6	4		
S. newport	14		1	5	4	4		
S. typlii	8		1	ប៉	1			
S. enteritidis	14		2 1 1	7	1 3 7	$\frac{2}{1}$		
S. pullorum	18	1	1	8 5		1		
S. gallinarum	16		1	5	7	3		
S. anatum	11				4	6		
S. gue	11				4	6		
S, newington	11			1	2	4		
S. senftenberg	10			1	4	4		
E. coli W	1			1				

examined. Results obtained with only one or 2 cultures of a given type are only a tentative indication of the susceptibility of that type to the substance. In general, it may be said that S. paratyphi A and S. typhi were quite susceptible to the action of streptomycin while S. pullorum and S. enteritidis were inhibited by relatively low concentrations. The remainder of the commonly occurring Salmonella types were decidedly more resistant. It is of interest that not only was S. enteritidis one of the more easily inhibited types, but the closely related forms, S. dublin, S. rostock, S moscow, and S. blegdam, were also inhibited by low concentrations of streptomycin

Summary. The in vitro inhibition of 412 cultures of Salmonella by streptomycin was

studied. All of the recognized types in the genus were included in the work. Of the commonly occurring types, S. paratyphi A and S. typhi were the most sensitive while S. pullorum and S. enteritidis were somewhat less susceptible The remainder of the types which are found frequently were more resistant The majority of the types required 2 to 4 times as much streptomycin to inhibit growth as did E. coli W. Different cultures of the same type often varied widely in their susceptibility to streptomycin

The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Kentucky Agricultural Experiment Station.

#### 15191

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Effect of Different Preparations of Streptomycin on Migration of Macrophages and Growth of Fibreblasts in Cultures of Normal Rabbit's Spleen.

			mats in Cult	ures of Nori	nai Kabbit'	s Spleen.		<del></del>
				Macrophage	s		Fibroblasts	
<u> </u>	Streptomyc	in	mean	f migration, oeular eter units		m	of growth, lean eter units	
Sample	Poteney, units in 1 mg	Dilution × 1000	Control cultures*	Test cultures	% difference	Control cultures	Test cultures	% difference
1	200	1:1 1:2 1:4	$269 \pm 63$ $179 \pm 9$ $179 \pm 9$	252 ± 42 192 ± 8 174 ± 4	- 6 + 7 - 3	755 ± 30 1112 ± 63 1112 ± 63	$433 \pm 21$ $968 \pm 52$ $731 \pm 66$	—13† —13 —34
2	242	1:2 1:4	$^{408}_{582} \pm ^{21}_{20}$	373 ± 11 567 ± 16	9 3	571 ± 31 960 ± 27	$\begin{array}{c} 433 \pm 26 \\ 863 \pm 58 \end{array}$	-24 -10
3	333	1:2 1:4	$     \begin{array}{r}       408 \pm 21 \\       582 \pm 20     \end{array} $	353 ± 9 550 ± 16	—13 — 5	571 ± 31 960 ± 27	396 ± 20 810 ± 46	31 16
4	270	1:2 1:4	$\begin{array}{c} 408 \pm 21 \\ 414 \pm 17 \end{array}$	$387 \pm 18$ $406 \pm 14$	— 5 — 2	571 ± 31 751 ± 54	377 ± 29 854 ± 50	-31 +14
5	218	1:2 1:4	$476 \pm 20$ $414 \pm 17$	382 ± 8 465 ± 20	20 +12	927 ± 55 751 ± 54	638 ± 30 784 ± 44	_31 + 4
6	102	1:2 1:4	$582 \pm 20$ $412 \pm 17$	$521 \pm 36$ $384 \pm 20$	—10 — 7	960 ± 27 846 ± 30	789 ± 36 827 ± 16	—18 — 2
7	220	1:2 1:4	$534 \pm 24$ $412 \pm 17$	$517 \pm 16$ $402 \pm 14$	— 3 — 2	843 ± 37 846 ± 30	655 ± 61 847 ± 28	-22 + 0.1
8	150	$1:2 \\ 1:4$	$534 \pm 24$ $412 \pm 17$	$521 \pm 21$ $392 \pm 14$	— 2 — 5	843 ± 37 846 ± 30	588 ± 47 804 ± 32	30 5
9	369	1:1 1:2	$\begin{array}{c} 467 \pm 22 \\ 467 \pm 22 \end{array}$	$430 \pm 14$ $448 \pm 17$	- 8 - 4	936 ± 37 936 ± 37	$722 \pm 39$ $982 \pm 61$	—23 + 5
10	190	1:2	$476 \pm 20$	$417 \pm 16$	-12	$927\pm55$	$662 \pm 15$	29
11	185	1:1 1:2	$468 \pm 17$ $468 \pm 17$	$506 \pm 15$ $480 \pm 11$	+ 8 + 3	805 ± 53 805 ± 53	732 ± 19 802 ± 27	-9 + 0.4
12	250	1:1 1:2	275 ± 11 275 ± 11	237 ± 8 265 ± 18	—14 — 4	852 ± 63 852 ± 63	812 ± 27 809 ± 38	5 5
13	336	1:1 1:2	429 ± 7 429 ± 7	$364 \pm 12$ $413 \pm 10$	—15 — 4	730 ± 23 730 ± 23	548 ± 28 609 ± 30	—25 —17
14	100	1:1 1:2	429 ± 7	353 ± 12	18	730 ± 23 730 ± 23	524 ± 23 691 ± 20	28 5

^{*} The value following the ± is the standard error of the mean.

of the volume of the final tissue culture clot. A similar amount of Tyrode's solution was added to extract used in the preparation of control cultures.

Cultures were placed in D-5 Carrel flasks and consisted of 0.5 cc of heparinized rabbit's plasma, 1.0 cc of serum-chick-embryo extract and 4 explants of spleen which were placed in

the medium before clotting occurred. Three flasks were prepared for each experimental condition. Preparations were incubated at 37°C for 5 days. Measurements of the migration of macrophages were made with an ocular micrometer at 96 hours of incubation by a method that has been described. Fibroblastic growth was determined on the fifth day

[†] Values in italies show significant variation from controls.

Precipitate obscured cellular migration.

by projectoscopic methods. The average radius of the growth or migration zone in each group of test cultures was compared with that of the corresponding controls and the results were expressed in terms of percentage of inhibition or stimulation.

Results. Samples of streptomycin obtained from different sources were uniformly low in cytotoxicity (Table I). A concentration of 1:2000 of streptomycin caused a slight but significant inhibition of migration of macrophages in 2 instances and a decrease of fibroblastic growth in 9 of the 14 samples tested. One sample of streptomycin in a concentration of 1:4000 caused a slight but significant inhibition of the growth of fibroblasts but a similar concentration of the other preparations of streptomycin tested did not inhibit growth. Fibroblasts were more sensitive than macrophages to streptomycin except in samples 12 and 14. In the case of sample 14 the drug formed a precipitate in the culture medium. Particulate material frequently has a more toxic effect on macrophages than on fibroblasts because the particles are engulfed by the wandering cells.

The results of tests with 2 samples of streptothricin are recorded in Table II. Both samples were much more toxic for fibroblasts than for macrophages. Leukocytes that were

TABLE II.

Effect of Different Concentrations of Streptothricin on Migration of Macrophages and Growth of Fibroblasts in Cultures of Normal Rabbit's Spleen.

Strept	othriein		
	Dilution	% variation f	rom controls
Sample	$\times$ 1000	Macrophages	Fibroblasts
1	1:0.5	100*	-100*
325 units	1:1	<b></b> 57	100*
per mg	1:2	45	100*
	1:10	- 4	100*
	1:50	+ 3	+ 2
2	1:20	+ 5	100*
345 units	1:40	+ 6	- 30
per mg	1:60	+ 5	- 28
	1:80	+ 1	- 7

^{* 100 =} complete inhibition of growth.

present in the migration zone at 24 hours showed about the same sensitivity to streptothricin as did macrophages. The highest concentrations of the 2 samples of streptothricin that were not toxic for fibroblasts were 1:50,000 and 1:80,000 respectively.

Comment. The low toxicity of streptomycin in tissue culture is in agreement with its relatively low toxicity for experimental animals.2-4,7-10 Recently Reimann, Elias, and Price14 have reported on the use of streptomycin in the treatment of typhoid fever. Serious toxic reactions due to streptomycin did not occur in their series of cases. Because all of the samples tested in the present study were relatively nontoxic, there was very little relation between the cytotoxicity observed and the potency of the preparation of streptomycin in units per milligram. It should be emphasized that the methods employed in the present investigation do not detect histamine activity, pyrogens or numerous other pharmacologic effects that do not interfere directly with the survival and growth of the cells being tested. The cytotoxicity of streptomycin is of about the same order as that of fairly pure penicillin tested by the same method.¹⁵ The relatively low toxicity of streptothricin for leukocytes and macrophages and the fairly high toxicity for fibroblasts demonstrate the advantage of observing the effect of the substance being tested on more than one type of cell. The deleterious effect of streptothricin on fibroblasts is in agreement with the toxicity of streptothricin for guinea pigs observed by Feldman and Hinshaw.

Summary. Several different preparations of streptomycin were tested on cultures of rabbit's spleen and were found to have a uniformly low toxicity for wandering cells and fibroblasts. Streptothricin had a relatively low cytotoxicity for leukocytes and macrophages but showed a fairly high cytotoxicity for fibroblasts.

¹⁴ Reimann, H. A., Elias, W. F., and Price, A. H., J. A. M. A., 1945, 128, 175.

¹⁵ Heilman, Dorothy H., unpublished data.

#### 15192

Physiological Observations upon Larval Eustrongylides. IX. Influence of Oxygen Lack upon Survival and Glycogen Consumption.

THEODOR VON BRAND AND W. F. SIMPSON.
From the Department of Biology, The Catholic University of America, Washington, D.C.

The larvæ of Eustrongylides ignotus are parasitic nematodes which lead in their natural habitat, encysted in Fundulus heteroclitus, a more or less completely aerobic life.^{1,2} In view of the fact, however, that many parasitic round worms have extremely well developed anaerobic functions, it appeared of interest to investigate some reactions of this helminth to the lack of oxygen. The influence of the latter on the survival of the worms and their glycogen consumption will be discussed in the following sections. All experiments were conducted under strictly sterile conditions; the technic used in isolating worms under aseptic conditions has been described previously.³

Survival Under Lack of Oxygen. It has been shown that larval Eustrongylides survive more than 30 months in vitro if kept at 20°C in rubber stoppered tubes containing sterile nutrient media. The two series upon which this statement was based have now come to an end and the results, not heretofore published, are summarized in Table I. That

TABLE I.
Survival of Larval Eustrongylides ignotus at 20°C in Sterile Media. Rubber-stoppered test tubes (25 x 150 mm) containing each 30 ce medium and one worm. Series A: Bacto-Broth 1.6%, NaCl 0.85%, glucose 0.5%. Series B: Bacto-Proteose-Peptone 1.0%, NaCl 0.85%, glucose 0.5%.

No.	of wo	rnis			rms liv ified mo	
at b	eginu	ing	36	42	48	50
Series		14	1	0		
,,	В	12	õ	3	2	0

some worms survived as much as 4 years outside of their host is quite remarkable in view of the fact that most parasitic worms die

rather rapidly when removed from their natural surroundings. Since the tubes containing these worms were tightly closed with rubber stoppers, it appeared likely that the gaseous atmosphere had undergone profound changes during the time of incubation. In the later stages of these series it became possible to analyze, in a van Slyke manometric apparatus, the gas present above the culture fluid after the worms had died. The average composition, in cases of larvæ having died after periods of 36 to 49 months after isolation, was 0.95% O2 (extremes 0.37 and 1.73%) and 2.63% CO2 (extremes 1.40 and 5.90%). These observations indicate that the worms tolerate a rather severe lack of oxygen.

The corresponding changes in atmosphere were more closely followed in another series in which the tubes were kept at 37°C. The same large size tubes were used as in the preceding series; the fluid medium consisted of 25 cc 1% peptone + 0.5% NaCl + 0.5% glucose and each tube contained only one worm. At the intervals specified in Fig. 1 4 worm tubes were used for analyses of the enclosed air. Despite the fact that the oxygen content diminished rather rapidly, falling below 1% after about 3 months, and that a pronounced accumulation of carbon dioxide occurred, all the worms were alive when the tubes were opened. It should be noted that the present data can only be used to show at what oxygen and carbon dioxide tensions the worms survive. They are insufficient to allow reliable calculations concerning the intensity of the gaseous exchange of the worms since control experiments showed that some changes in the atmosphere occur also in tubes containing no larva. These changes are probably due in part to the spontaneous oxidation of some constituent of the medium, in part to the oxidation of substances contained in the stopper.

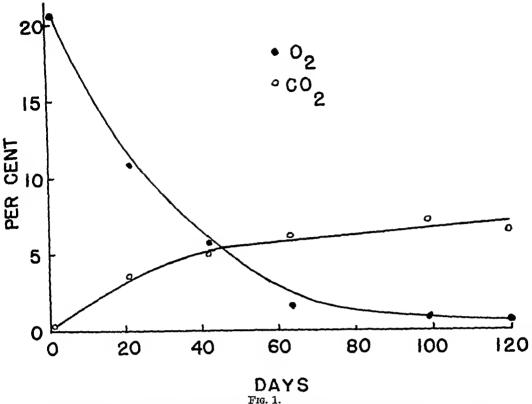
While the worms are obviously not damaged

¹ von Brand, T., J. Parasitology, 1938, 24, 445.

² von Brand, T., Biol. Bull., 1942, 82, 1.

³ von Brand, T., and Simpson, W. F., Proc. Soc. Exp. Biol. And Med., 1942, 49, 245.

⁴ von Brand, T., and Simpson, W. F., J. Parasitology, 1944, 30, 121.



Oxygen and carbon dioxide in the atmosphere enclosed above the culture fluid in tubes containing larval Eustrongylides during incubation at 37°C.

by relatively low oxygen tensions, they do asphyxiate under complete lack of oxygen. A series of 30 was set up in standard size test tubes almost completely filled with Brewer's thioglycollate medium (Infusion from 37.5 g meat per 100 cc, 1% thio-peptone, 0.5% NaCl, 0.5% glucose, 0.2% dipotassium-phosphate, 0.1% Na-thioglycollate, 0.05% agar, 0.0002% methylene blue, initial pH 7.5) which were tightly closed with rubber stoppers. anaerobic conditions actually prevailed is indicated by the fact that the methylene blue remained completely reduced throughout the course of the experiment. The average survival of these worms was 18 days (extremes 4 and 25 days). It now became necessary to ascertain whether the brief survival was actually due to the lack of oxygen, or to a toxic action of some constituent of the medium. To this purpose a new series was established in test tubes filled only to about one-third with the above medium and these tubes were closed with cotton stoppers only. In all 21 tubes employed the movements of the individual worm was sufficient to mix the contents to such an extent that enough oxygen diffused from the surface to oxidize the methylene blue throughout the entire column within 2 days. The average survival of these larvæ was 98 days (extremes 10 and 161 days). The difference between the 2 series proves that the ingredients of the medium are non-toxic and not responsible for the early death of the anaerobically kept worms.

Influence of Anaerobiosis on Glycogen Consumption. In order to study the influence of lack of oxygen on the glycogen consumption of the larvæ, glycogen determinations according to von Brand's modification of Pflueger's method were carried out on freshly isolated worms and on worms kept for 6 days at 37°C

⁵ von Brand, T., Skandin. Arch. Physiol., 1936, 75, 195.

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NaCl 0.85%	7	51	53	80.8	6.7	-		, ' &		1		- 1			el	GI		IId	
Peptone 0.5% + 0.2% glucose	न्यू। न	e 10	8	7.81	6.5	14 ~ <b>3</b> 4	:	: :! E	6.8 - 2.4			7				Ι'	4.3	£.9.4	
Peptone 0.5% + 0.5% NaCl	ħ	Ť.	S S	6.61	0.5	~∱+	50	69						5.0	4. ċ	4.0	7.41	7	
ose W Brandse	7	51 4	38	7.11	0.5	-1	96	99								•	5.0		
	} {	!					) i	3	0.0	1	0.96		9; 7	09	5.5	6.7	5.1		
													-	-				;	

in various solutions under aerobic and anaerobic conditions respectively (Table II). The test tubes were not closed by a stopper or cotton plug, but their sterility was maintained by placing over their open end an inverted loosely fitting glass vial, thus allowing an easy interchange with the surrounding atmosphere. Anaerobic conditions were established by placing the rack supporting the tubes into a desiccator in which the oxygen was absorbed by an alkaline solution of pyrogallol (Buchner's procedure). The completeness of the absorption was ascertained by withdrawing a gas sample and analyzing it in a van Slyke manometric apparatus.

The results summarized in Table II show that, under aerobic conditions, the glycogen consumption was greatest in pure saline, indicating that the nutritive material contained in the other series exerted a glycogen sparing This confirms earlier observations extending over longer periods.4 Under anaerobic conditions, on the other hand, no such difference could be observed. The worms are apparently unable to utilize either peptone or sugar contained in the medium when oxygen is completely lacking. The evidence for this assumption is perhaps not quite convincing; direct determinations in the medium were not carried out, because the evaporation taking place under the condition of the experiments excludes the possibility of gathering reliable data. The glycogen consumption itself under anaerobic conditions was about three times that found under aerobic conditions, if for such comparison only the glycogen disappearance in a non-nutritive medium is used. This, of course, is due to the fact that an anaerobic carbohydrate breakdown yields but little energy.

The pH of all the solutions employed decreased during the experiments and this decrease was much more pronounced under anaerobic than aerobic conditions. This is in agreement with the previously reported excretion of acids by anaerobically kept Eustrongylides larvæ.¹

A number of comparable series were carried out at 20°C. Despite the fact that they extended over 3 weeks each no definite conclusions could be reached. At this temperature

the daily rate of aerobic glycogen consumption is very low⁴ and consequently the variations in glycogen content between various batches of worms may falsify any result. It was unfortunately not possible to conduct the experiments over a longer period, because too many worms die after 3 weeks anaerobiosis.

Summary. Some specimens of larval Eustrongylides ignotus could be maintained

for 4 years in vitro. The worms survived for long periods in media showing a pronounced partial lack of oxygen, but they did not tolerate complete absence of oxygen for very long periods. Anaerobically kept worms seemed not to feed but they consumed from their body reserve about three times as much glycogen as worms starving under aerobic conditions.

### 15193 P

Effect of Acute A-V Fistula on Circulation Time and Auricular Pressure in Dogs.

E. C. HERINGMAN, H. A. DAVIS, AND J. D. RIVES.
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The physiological effects of A-V fistula have long been the subject of extensive experimental investigation. Added impetus has been given to this work by successive wars. The existence of such effects as Nicoladoni-Branham phenomenon, increased pulse pressure, decreased diastolic pressure, and the relationship of these changes to the size and location of the fistula, have been definitely established. On the other hand, another group of physiological effects or the explanation thereof, such as increased cardiac output,1,2,3 increased blood volume, and increased pressure in the large veins proximal to the fistula,4.5 have been matters of considerable controversy. Finally there are some effects which have been studied incompletely or not at all. The present investigation was undertaken to determine the effect of A-V fistula, first, on femoral and jugular circulation times and the relationship between the two, and second, on auricular pressures.

Method. Thirty observations were made

on 5 dogs. The animals were anesthetized with nembutal intravenously, 1 grain per 5 lb of body weight. The right femoral artery and vein, and right jugular vein were exposed under rigid aseptic conditions. Right femoral arterio-venous fistulæ were made in the manner described by Holman.4 Femoral and jugular circulation times, using the cyanide method described by Robb and Weiss,6 were determined before and after the fistula was made. All injections into the femoral vein were made at a point just proximal to the site of the fistulous opening into the vein. The injections into the jugular vein were made at a point midway between the lower edge of thyroid cartilage and sternum. Next, auricular pressures were determined with the fistula open and closed. A No. 10 French catheter, with tip cut off, was introduced into the right auricle by way of the right jugular vein according to the method of Richards et al.7 Heparin was added to the saline in the catheter to prevent clotting. In one dog (No. 5) auricular pressures were not studied until 5 months after the fistula was made. All readings were made in duplicate and mean readings were determined.

¹ Lewis, T., and Drury, A. N., *Heart*, 1923, 10, 301.

<sup>Ellis and Weiss, S., Am. Heart J., 1929-1930, 5.
Grollman, A., Cardiac Output, Hopkins, 1932.</sup> 

⁴ Holman, E., Arterio-venous Ancurysm, Macmillan Company, 1937.

⁵ Reid, M. R., and McGuite, J., Annals Surg., 1938, 108, 643.

⁶ Robb and Weiss, S., Am. Heart J., 1932-1933. S.

⁷ Richards, Cournand, Darling, Gillespie, and Baldwin, Am. J. Physiol., 1942, 136, 115.

TABLE I.

		Femor	al circula	ion time	s		Jugular	circulatio	n times	
	Before f	istula	Afterf	stula		Before fi		After fi		
Dog No.	Readings	Mean	Readings	Mean	Dift. + or —	Readings	Mean	Readings	Mean	Diff. + ar~
ទ	9.8 9.0	9.4	6.5 6.9	ij.7	2.7	6.2	6.2	8.8 8.0	8.5	+2,3
17	8.0 7.8 8.0	7.9	6.0 6.8 6.6	6.5	1.4	6.3	6.5	8.7 7.5 7.5	7.5	+1.0
18	8.4 8.0	8.2	5.6 5.6	5.6	2.7	7.3 7.7	7.3	7.2 7.0	7.1	4
10	7.5 7.5	7,3	5.2 4.5 4.5	4.7	-2.8	7.0 6.4 5.8 5.8	6.0	5.1 5.3	5.2	8
20	9,8	9,9	7.0 6.9	7.0	2.9	9.8 8.2	8.0	7.9 8.1	· S.0	±0
Mean Standard	8.52		6.0			6.9		7.3		
deviatio 't'' P.	n ±0.826 6.3 <0.01 nificant diff	erence	±0.777			±1.267 0.81 >0.4 No significa	nt diff	±1.338		

Results. Studies of the femoral circulation times showed that a statistically significant speed-up of circulation occurred when a femoral arterio-venous fistula was produced in the vascular tree. The mean reduction in femoral circulation time in the 5 dogs was 2.64 seconds or 30.9%. On the other hand, jugular circulation times gave varying results. A-V fistula in 2 dogs caused a mean increase of the jugular circulation time amounting to 1.63 seconds. In 2 dogs it caused an insignificant decrease of the jugular circulation time, and in one dog no change was observed. (Table I.)

In the 5 dogs there was a rise in auricular

TABLE II.

Mean Auricular Pressure—CMs H₂O in Experimental A-V Fistulæ.

Dog No.	Fistula elosed	Fistula open	Difference
17 18 19 20 Mean Standard deviatio	4.7 3.5 6.2 3.7 4.5 n ±1.06 3.2 <0.05 erence is signif	8.8 0.4 9.3 6.4 7.7 ±1.33	+4.1 +2.9 +3.1 +2.7
	-		

pressure when the fistula was open. The mean increase in pressure amounted to 3.2 cm of H₂O, or a rise of 71.1%. (Table II.)

The measurements of auricular pressures obtained in Dog No. 5 were not included, because no control readings were obtained when the fistula was first made. The rise in pressures in the 4 dogs with acute fistulæ was statistically significant.

Discussion. The results indicate that the introduction of a femoral A-V fistula into the circulation will cause the following two important changes: first, speed-up of circulation in the fistula circuit, and second, an increase in auricular pressure. These two observations together suggest that a greater volume of blood is being brought to the right auricle during a given period of time. Therefore, if the heart is not failing, it must put out more blood in the same given period of time. This provides indirect evidence that an A-V fistula causes an increase in cardiac output.

On the other hand, statistical analysis demonstrated that the circulation time in the jugular circuit showed no significant change. It is known that the jugular circulation time is practically a measurement of the rate of

flow through the pulmonary circulation to the carotid sinus. Since the jugular circulation time did not change significantly, it is obvious that the increase in rate of blood flow occurred primarily in the segment of the fistula circuit extending from the fistula to the heart.

The significant increase in auricular pressure caused by an A-V fistula seems to substantiate the work of Holman, who maintained that there was an increase of pressure in the proximal venous portion of a fistula circuit.

#### 15194

### Value of Sunflower Seed Protein.

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From statements of a recent report, the nutritive value of sunflower seed meal would appear to depend more on its content of B-complex vitamins than of protein, even though 53% of the product studied was protein.1 In a study of the biological value of the protein of sunflower seed meal by the nitrogen balance method, it was concluded that this protein is inferior to that of soybean meal, and is "in the same class as the better cereals oats, wheat, and barley."2 reports indicate that sunflower seed meal is of only mediocre value as a source of protein. On the other hand, the amino acid analysis of sunflower seed meal compares very favorably with analyses of beef muscle and other tissue proteins of high value.^{3,4} On the basis of known chick requirements,5 the meal should be adequate as a sole source of protein with the possible exception of a slight deficiency of The obvious disagreement between lysine. the biological and analytical data led us to conduct further tests with the chick.

Two-week-old White Leghorn chicks were segregated into groups on the bases of weight and gain during the second week after hatching, and were given the experimental diets, which were (except for the protein sources) identical with those previously used. 6,7,8 All the protein (20% of the diet) was supplied by a South American sample of sunflower seed meal which contained 46.7% protein (N × 6.25) or by a laboratory-prepared meal which contained 39.9% protein. Supplemented casein and deboned sardine meal served as positive controls in these experiments.

The data on sunflower seed meal presented here (Table I) indicate quite clearly that the value of this product for chick growth and efficiency of feed utilization is very high. It compares favorably with other recognized complete sources of amino acids for the chick. In fact, the protein of sunflower seed meal is the most complete vegetable protein for chick growth that we have studied. This result is in agreement with the amino acid analyses of the protein^{3,4} and the known chick requirements.⁵ It is apparent that even lysine is present in adequate amount, although lysine is deficient in many cereal and oilseed meal proteins.

¹ Day, A. G., and Levin, E., Science, 1945, 101, 438.

² Mitchell, H. H., Hamilton, T. S., and Beadles, J. R., J. Nutrit., 1945, 29, 13.

³ Block, R. J., and Bolling, D., Arch. Biochem., 1945, 6, 227. Also earlier private communications from R. J. Block.

⁴ Grau, C. R., and Almquist, H. J., Arch. Biochem., 1945, 6, 287.

⁵ Almquist, H. J., Trans. Am. Assn. Cereal Chemists, 1945, 3, 158.

⁶ Grau, C. R., and Almquist, H. J., Proc. Soc. Exp. Biol. and Med., 1944, 57, 187.

⁷ Almquist, H. J., and Grau, C. R., Poultry Sci., 1944, 23, 342.

⁸ Grau, C. R., and Almquist, H. J., Poultry Sci., 1944, 23, 486.

Comparative Gains on Chicks on Diets Containing the Equivalent of 20% Crude Protein Exclusively from
Sunflower Seed Meal, Sardine Meal, or Casein Plus Certain Amino Acids.

Exp. No.	Amino acid source	Chicks po group, No.	Duration of exp., days	Avg total gain,		Gain per g feed consumed
1	60		cap, anjs	g	%	g
1	Sunflower seed meal, com'l.	5	11	62	5.3	
	Sardine meal*			67	5.7	
2	Same	6	12	121	= =	0.02
		U	15	112	$\begin{array}{c} 5.7 \\ 5.4 \end{array}$	$\begin{array}{c} 0.36 \\ 0.34 \end{array}$
				11.5	0.2	0.34
3	Sunflower seed meal, com?.	10	21	147	4.8	0.38
	Casein, arginine, cystine, glycine			147	4.8	0.39
4	Sunflower seed mealt	10	9	76	6.5	0.42
	Sunflower seed meal, t lysine		b	80	6.5	0.43
	Casein, arginine, cystine, glycine			57	5.5	0.43

^{*}This product was deboued by floating on carbon tetrachloride; it contained 85.0% protein (N  $\times$  6.25). †Sample prepared in the laboratory from sunflower seed.

The amino acids which are required by the chick for optimal growth are greater in number and equal to or greater in quantity than those required by the rat.⁵ The disagreement between the rat biological data and the amino acid and chick biological data may be due to

the use of poor quality samples in the rat

Summary. The protein of sunflower seed is a complete single source of amino acids for the growth of the young chick, when fed to provide 20% protein in the diet.

## 15195

## "Thermostable" Thromboplastin from Human Placenta and Chicken Brain.*

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In the course of a study of the hormones of placenta we observed that extracts of this organ exhibit a strong blood-coagulating action. The active substance resembled thromboplastin, the factor which by converting prothrombin into thrombin activates the reaction fibrinogen  $\rightarrow$  fibrin and thus promotes the coagulation of blood. It could be shown that this active agent is relatively thermostable.

Heat stable thromboplastin from placenta is of special therapeutic interest, since its use makes it possible to avoid the side-effects which attend the local or parenteral administration of thromboplastin from heterogenic source.^{2,3} Since thromboplastin of birds is less

active on mammal blood than is mammal thromboplastin and vice versa, it seems probable that the two are different. A comparison between these two thromboplastins was therefore carried out.

The properties of thromboplastin have been studied in the last few years by various laboratories. A very extensive research has been

^{*} Generously aided by a grant from the Rocke feller Foundation,

¹ Finkelstein, M., Nature, 1945, 155, 202.

² Eley, R. C., Green, A. A., and McKhann, C. F., J. Pediat., 1936, 8, 135.

³ Edsall, Y. T., Ferry, R. M., and Armstrong, S. H., J. Clin. Invest., 1944, 23, 557.

undertaken by Chargaff et al.⁴⁻⁸ These authors isolated substances of a thromboplastic nature from animal tissues by a variety of methods. Using water as extracting medium and purification of the extract by differential centrifugation procedures they obtained thromboplastic preparations described as "high molecular lipoprotein." By means of organic solvents thromboplastic preparations belonging to the phosphatide group were separated. Both preparations proved relatively thermostable.^{5,6}

Experimental. Preparation of Thromboplastin from Placenta. Placenta was perfused with tap-water and then minced with sand. 20 g of the mince was extracted with 20 cc cold distilled water. The suspension was centrifuged. The supernatant fluid was sucked through a Seitz filter, and the filter pad was then washed with distilled water until the wash-water became colorless. The filter pad was broken up under cold distilled water and the suspension was filtered through filter paper. The resulting almost colorless, opalescent filtrate contained the thromboplastin used in the present study. It contained 1.4 mg dry matter per cc and exhibited a protein reaction with sulfo-salicylic acid.

Preparation of Thromboplastin from Chicken Brain. Twenty g of chicken brain was freed from grossly visible blood vessels and minced in a mortar with sand in 20 cc of water. The mash was pressed through gauze. After centrifugation the supernatant was diluted with equal parts of distilled cold water and was centrifuged again. The upper layer was removed with a pipette and filtered through a Seitz filter. Subsequent steps were as described for the preparation of thromboplastin from placenta. The resulting solution contained 1 mg dry matter per cc.

The thromboplastic activity of the preparation from placenta was tested on oxalated human plasma, and the preparation from chicken brain was tested on native† chicken or rooster plasma. (The thromboplastin from chicken brain shows very little activity when tested on human oxalated plasma, and high activity when tested on chicken plasma; thromboplastin from human sources exhibits the reverse behavior.)

The "coagulation time" of oxalated human plasma was tested by the addition of 0.1 cc thromboplastin to 0.1 cc plasma. After 5 minutes of incubation at 37°C 0.1 cc 0.02 M calcium chloride was added and the tube was shaken vigorously. The time of clot formation was recorded with a stop watch.

For the determination of the "coagulation time" of chicken plasma 0.1 cc of chicken plasma was transferred to a small clean tube kept in a water bath at 37°C. After 5 minutes of incubation 0.05 cc of thromboplastin was added with a micropipette and the tube was shaken vigorously. The time from the addition of the thromboplastin until clot formation was noted with a stop watch. Every experiment was performed at least 3 times. Only plasma specimens whose coagulation times without thromboplastin were not less than 2 hours were used.

Results. As we have reported in a preliminary paper¹ the purified aqueous placenta extract with strong thromboplastic activity is not markedly affected by heating for 5 minutes in a boiling water bath. This holds true, however, only in tests carried out with fresh oxalated human plasma. When the experiment is performed with plasma stored for some days in a refrigerator, the coagulation time in the presence of heated thromboplastin is greately increased in comparison to that found with an unheated preparation (Table I).

The thromboplastic preparation from chicken brain is thermostable in the sense that it withstands heating in a boiling water bath for 5 minutes, but it differs from thromboplastin from placenta in some other respects. Preparations of thromboplastin from placenta

⁴ Chargaff, E., Moore, G. H., and Bendich, A., J. Biol. Chem., 1942, 145, 593.

⁵ Chargaff, E., J. Biol. Chem., 1944, 155, 387.

⁶ Chargaff, E., Bendich, A., and Cohen, S., J. Biol. Chem., 1944, 156, 161.

⁷ Cohen, S., and Chargaff, E., J. Biol. Chem., 1940, 136, 243.

⁸ Cohen, S., and Chargaff, E., J. Biol. Chem., 1941, 140, 689.

th Chicken or rooster plasma may be kept liquid without addition of a decaleifying agent if drawn carefully and stored in a clean container in a refrigerator.

TABLE I.

Effect of Heated and Unheated Thrombophastin on
Congulation Time of Fresh and Stored Plasma.

	Congulation time in sec.						
Age of plasma in days	Unheated thromboplastin	Heated thromboplastin					
0	12	12					
0	13	13					
0	15	18					
2	. 18	23					
3	21	28					
7	42	185					
28	70	> 10 min.					

were affected very slightly or not at all by heating. The activity of the heated preparation remained constant when the preparation was kept in a refrigerator. The chicken brain preparations, on the other hand, were affected much more strongly by heating. The coagulation time of heated brain preparation was in some cases about twice that of the unboiled preparation. If, however, a heated preparation was kept in the cold for some days and then tested, its original thromboplastic power was found to be completely restored (Table II).

TABLE II.
Renctivation of Heated Thrombophastin from Chicken Brain Kept at 4-6°C.

Time between heating of	Congulation	time in sec.
thromboplastin and test performed	Unheated thromboplastin	Heated thromboplastiu
Immediately	28 28	52 30
1 day 7 days	26	28

Enzyme chemistry knows similar cases. If, for example, trypsin is inactivated by heating for a short time, reactivation of the enzyme on cooling is complete.⁹

Native chicken or rooster plasma shows no prolongation of coagulation time after storage in a refrigerator for a considerable period of time (1 month) when tested with boiled and reactivated or unboiled chicken brain thromboplastin (Table III). On the other hand, oxalated human plasma, as stated previously, shows when stored a prolongation of its coagu-

lation time as determined on a boiled preparation of thromboplastin from placenta.

Discussion. This paper shows that the "coagulation time" of stored oxalated plasma is greater in the presence of heated thromboplastin than in the presence of unheated thromboplastin. On the other hand, the "coagulation time" of fresh oxalated recalcified plasma or stored "native plasma" (chicken plasma) is the same for heated and unheated thromboplastin.

TABLE III.

Effect of Heated and Unheated Thromboplastin
from Chicken Brain on Stored Chicken Plasma.

	Coagulation time in sec.						
Age of plasma, days	Unheated thromboplastin	Heated thromboplastin					
0	30	31					
1	29	31					
3	31	30					
30	29	32					

An explanation of this phenomenon is suggested by the findings of Quick. Quick believes that prothrombin is composed of two components A and B, which are bound by calcium and thus protected. In decalcified (oxalated) plasma the component A undergoes destruction and gradually disappears from the plasma. "Native" plasma, however does not change in its prothrombin content on storage as it is protected by calcium.

In view of these and our own findings, we assume that thromboplastin too is composed of two components, I and II. One of these is thermostable (I) and withstands heating for 5 minutes, while the other (II) undergoes destruction. The thermostable component (I) acts on plasma in which the prothrombin complex (parts A and B) is still intact, e.g., on fresh oxalated plasma or on "native" plasma (chicken plasma), but does not act on plasma in which the component A of pro-(stored oxalated thrombin is destroyed The two components (I and II) of thromboplastin together, i.e., in an unheated thromboplastic preparation, exhibit activity towards stored oxalated plasma.

⁹ Sizer, I. W., Advances in Ensymology, 1943, Vol. III, 35-60.

¹⁰ Quick, A. J., Am. J. Physiol., 1943, 140, 212.

other words, they activate the component B of prothrombin.

It may be concluded, therefore, that the thermostable part of thromboplastin (I) is responsible for the activation of component A of prothrombin. The component B may be activated either by the two parts of thromboplastin together, or by the thermolabile one alone, but the mechanism of activation of component B has not yet been ascertained.

The observation of certain workers that thromboplastin is definitely destroyed by heat may be explained by the fact that the behavior towards heat depends on the method of the preparation. It seems possible that inactivation is due to presence together with the thromboplastic factor of proteins which by denaturation eliminate the active agent.

Since our thromboplastin preparation is thermostable in respect of its ability to coagulate fresh plasma or blood, it is particularly well suited for diagnostic and therapeutic use. It has been used by us for the following purposes: (1) To determine prothrombin time. The aqueous solutions are set up in ampoules for use as necessary; (2) To obtain local stoppage of blood flow; (3) To fix skin transplants. The titrated plasma is placed over the wound together with thromboplastin and calcium and the clot which forms within a few seconds serves as an adhesive for the skin graft (Sano¹¹). The advantages of our preparation are its availability in an ampoule, and the fact that it contains human protein. (The undesirable possible by-effects of the use of non-human protein are thus eliminated.) Our clinical experience of this technic will be discussed jointly with Prof. Mandl elsewhere.

(4) For injection into man. Since the preparation contains homologous protein undesirable reactions are avoided. Experiments on this aspect are under way.

A sterile and thermostable thromboplastin from chicken tissue may prove useful in plasma culture. The preparation from chicken brain elicits coagulation of chicken plasma within a few seconds. The thromboplastin can be heated and in this way accompanying growth-promoting substances can be The coagulation of plasma in destroyed. tissue cultures is usually induced by the addition of fresh chicken embryo extract which may contain growth-promoting substances to the culture medium. Research on the effect of growth-promoting substances in tissue cultures has been complicated by this circumstance.

Summary. A method of preparation of thermostable thromboplastin from human placenta and of thermolabile but reactivable thromboplastin from chicken brain is de-The properties and differences of these preparations are discussed. Human placenta thromboplastin is not inactivated by heating at 98°C for 5 minutes. Chicken brain thromboplastin is inactivated by the heat treatment but is subsequently reactivated when it is stored at 4-6°C. The different activities of heated and unheated placenta and chicken brain thromboplastin on stored oxalated human plasma and native chicken plasma are explained by the assumption that thromboplastin is composed of two factors. one of them thermostable and the second thermolabile. Clinical, surgical, and research applications of thermostable preparations of thromboplastin are discussed.

¹¹ Sano, M. E., Am. J. Surg., 1943, 61, 105.

## An Improved Cage for Nitrogen Studies with Mice.

M. L. MACLACHLAN AND L. A. MUNRO. (Introduced by R. G. Sinclair.)

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During a study of nitrogen balance in mice on different diets, it was found that the experimental arrangements described in the literature for similar experiments were not entirely satisfactory. In such studies the total nitrogen ingested and excreted must be determined. Spillage of food, fouling of the food in the container, loss of nitrogen from excreta via ammonia and poor ventilation are some of the factors contributing to error in such determinations.

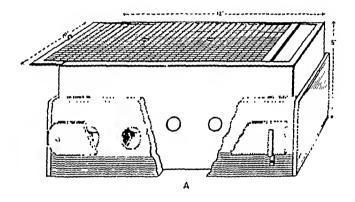
The cage described by Bittner1 is not suit-

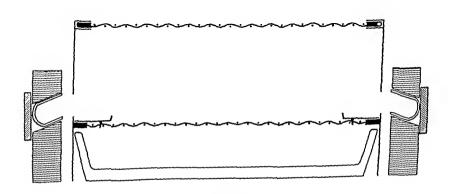
able for this investigation, particularly with the diets used in our experiments.

In our first experiments, beaker cages similar in principle to the glass containers described by White² were used. The feeding dishes were equipped with screw lids having a small orifice and were held in position by metal collars attached to the floor. In spite

¹ Bittner, J. J., Chapter 13, Biology of the Laboratory Mouse, G. O. Snell, editor, Blakiston Company.

2 White, F. R., Cancer Research, 1945, 5, 265.





A. Exterior aspect of feeding cage.
B. Cross-section showing glass collecting tray.

of these precautions food spillage invariably Where nitrogen intake is determined from the weight of food eaten or where the caloric intake is being followed, spillage may be a large source of error. It was found that the individual cages used by White or by ourselves gave incomplete separation of the urine and feces. Difficulty was also experienced in cleaning the fine screens. Poor ventilation is evident when the feces are not removed daily. Loss in ammonia, however, was reduced by putting 25% sulphuric acid in the bottom of the mouse jar. It was observed that mice kept in individual cages ate less and did not maintain their weight as well as mice kept together in larger cages. Similar observations have been made by Brunschwig et al.3

To overcome the objections inherent in these methods of management of the mice, the metabolism chamber shown in Fig. 1 was devised. It was designed to accommodate 10 mice. It consists of a metal cage 12" x 10" x 5" with a single floor of No. 3 gauge screen, 2 inches from the bottom. This fits over a glass tray. Five feeding holes, 5%" in diameter, are in each of 2 opposite walls. These holes are opposite recesses in a 1-inch board which forms part of an outer frame into which the metal cage fits, but which is offset 3%"

from the metal wall. The recesses, %" in diameter, are depressed at an angle of 20° and are receptacles for the glass food cups. A wooden gate acts as the back-stop and enables the food cups to be inserted one at a time. The glass food cups are cut to fit the oblique hole so that there is a space of 3/16" between the edge of the cup and the wall. Inside and touching the wall below each set of feeding holes is a movable metal tray kept in a position by 2 metal projections into the screen floor. A sliding screen cover furnishes adequate ventilation. Water bottles are inserted through the screen mesh. The whole assembly sits on a sheet of clean paper.

This experimental set-up solves the problem of spillage and fouling of the food. The mouse can reach the bottom of the feed cup but does not pull food into the cage. Any food pulled out of the cup drops between the metal wall and the wooden frame to the clean paper. Occasionally some food may stick to the head of the animal and subsequently be dislodged when the mouse again pokes its head into the feed hole. This material drops to the metal tray, and is recovered. Spillage was reduced to a minimum when the diet had the consistence of toothpaste.

Summary. A cage for the proper management of mice during nitrogen balance studies is described. The arrangement eliminates spillage of food, fouling by excreta, loss of ammonia, and other sources of error.

#### 15197 P

## Autoantibodies in Rheumatic Fever.*

PHILIP A. CAVELTI. (Introduced by K. F. Meyer.)

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As reported previously^{1,2,3} autoantibodies to kidney can be produced by immunization of animals with mixtures of group A streptococci and kidney of the same species. The streptococci thereby confer antigenicity on renal material which, as such, is nonantigenic in the homologous species. The autoantibodies to kidney thus formed react specific-

ally with plain normal homologous kidney as demonstrated in vitro by the collodion par-

³ Brunschwig, A., and Rasmassen, R. A., Cancer Research, 1941, 1, 371.

^{*} This investigation was aided by grants from the Commonwealth Fund.

¹ Cavelti, Philip A., and Cavelti, Else S., Arch. Path., 1945, 39, 148.

² Idem., Arch. Poth., 1945, 40, 158.

³ Idem., Arch. Path., 1945, 40, 163.

ticle technic. Their in vivo effect is the production of glomerulonephritis by means of their reaction with the immunized animal's own kidney.

It is thus conceivable that human glomerulonephritis also might be due to the pathogenic action of specific nephrotoxic autoantibodies produced as a response to an antigen resulting from a combination of streptococcal and renal material during the streptococcal infection, which commonly precedes glomerulonephritis by about 2 weeks.

Investigations as to whether substances from other homologous tissues, besides kidney, also might be rendered antigenic by combination with streptococci, and as to the lesions resulting from such autoantibodies, are under way and will be reported later; however, it can be stated that autoantibodies to heart, skeletal muscle, and connective tissue can be produced in this manner.

These results together with the fact that, similar to glomerulonephritis, rheumatic fever also is preceded by a streptococcal infection, suggest the possibility that autoantibodies to certain tissues might play a role in this disease.

Attempts were undertaken to demonstrate in the serum of patients with acute rheumatic fever, autoantibodies to human heart, the organ most regularly affected by this disease. The purpose of this paper is to present a short report on the results obtained so far in this phase of the studies.

Material and Methods. The antigens employed were saline extracts of human heart. Control serums were obtained (a) from normal persons and (b) from blood samples taken for routine laboratory tests from patients of the University of California Hospital in San Francisco. These controls therefore represent a diverse and unselected group of patients. The sera of patients with acute rheumatic fever were obtained from various local sources.

As serologic method the collodion particle technic⁴ was employed. Appropriate constant amounts of collodion particles sensitized with extract of human heart were added to a series of tubes containing progressive dilutions of

the serum to be tested. After an incubation of one hour at room temperature, the tubes were centrifuged for 3 minutes at 1,400 revolutions per minute. The results were read while the tubes were carefully shaken. Agglutination was recorded from plus-minus to 4-plus. In tubes in which no agglutination had occurred, the collodion particles could readily be resuspended smoothly, so that no particles could be seen by the naked eye.

Results. Antibodies to human heart were demonstrated in the majority of the samples tested from patients with active rheumatic fever. The titers, in terms of serum dilutions, varied between about 1:40 and 1:320. The serums of 36 patients have been tested so far. Strong positive reactions (2-plus or stronger) were recorded in 20 of these patients; weak, but definitely positive tests were obtained in 7 cases, and the remaining 9 patients showed doubtful or negative reactions.

From many cases repeated samples of serum were obtained at intervals of 2 to 5 weeks. Thus the total number of serums taken from patients with rheumatic fever tested so far is 67; of these, 47 gave definitely positive reactions.

The most satisfactory antigen was obtained from the heart of a case of postoperative pulmonary embolism. Extracts from 3 other human hearts, however, also gave positive reactions with serum of rheumatic patients.

Controls. (a) The serums of 12 normal persons all gave completely negative results; (b) of serums from 84 patients with various diseases, one (a case of chronic myeloid leukemia) was weakly positive and three others gave a doubtful reaction; the remaining 80 failed to give any serologic reaction with human heart.

Comment. From the results obtained so far it appears that, in general, these auto-antibodies to heart are present during the early and most active stages of the disease and disappear when the rheumatic process becomes inactive. Thus most, if not all, of the cases which gave negative serologic tests seemed to be well on the way to recovery. On the other hand, a few cases have been observed which gave particularly strong serologic reac-

⁴ Cavelti, Philip A., J. Immunol., 1944, 49, 365.

tions with human heart and in which the reaction persisted, although quantitatively decreasing, up to a time when the rheumatic process seemed to have become inactive clinically.

Studies on a larger scale on the various aspects of the serologic phenomenon described

are under way and will be reported upon completion.

Summary. From the results reported it would seem that the presence of autoantibodies to human heart has been demonstrated in 75% of a group of patients with acute rheumatic fever.

## 15198

## Recuperation from the Effects of Tenotomy on Neuromuscular Transmission.*

P. THOMSEN AND J. V. LUCO.

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Thomsen, Altamirano, and Luco¹ demonstrated the following changes, related to transmission in muscles 3 to 15 days after transection of their tendons: (a) greater development of tension in the third stages of muscular contraction relative to the first (see Rosenblueth and Cannon²); (b) diminished or abolished post-tetanic potentiation; (c) decreased sensitivity to curare; (d) more intense post-tetanic decurarization. We believed it would be of interest to study neural transmission in muscles which had undergone tenotomy but which had spontaneously re-established continuity of the tendon.

Method. The experiments were conducted on the soleus muscle of the cat 1.5 to 4 months after section of the Achilles tendon. In each study the responses of the operated muscle were inscribed on a drum, the homologous muscle of the opposite extremity being used as a control. Each tibia was fixed with clamps and the tendon attached to the short arm of a lever which exerted traction on elastic bands. The popliteal nerve was stimulated. Curare Merck was administered intravenously.

Results. The findings can be grouped into 3 separate categories: In the first, in which 3 cats were studied, 4, 4, and 3 months after tenotomy, the walk was the same as in recently

tenotomized cats, re-establishment of the tendon not having occurred. In these the muscular atrophy was marked and the development of tension so slight that detailed studies were not carried out. In the second series 3 cats 4, 1.5, and 1.5 months after tenotomy were studied. In all of them the walk was improved and the tendon showed a greater degree of re-establishment than in the first group. Response to the stimulation of the nerve with high frequencies (250-500 per second) was equal to that of normal muscle: however, the graph was more like that of a recently tenotomized subject in responses to lower frequencies, in post-tetanic potentiation, and in sensitivity to curare. In the third series in which 4 cats of 4, 3, 3, and 1.5 months after tenotomy were studied, the walk was normalized and the tendon has practically re-established its former status. The responses to all frequencies of stimulation were within normal expectations. Post-tetanic potentiation was present in only 2 of the 3 cases studied. The sensitivity to curare had not only returned fully, but was actually in excess The tension developed by the muscle with re-established tendon is only onehalf to one-third that of normal muscle.

Discussion. The observations made in this study reveal that it is possible to obtain a restoration of the normal function of neuromuscular transmission altered by transection of the tendon. This restoration does not occur in an abrupt form; that is, the various changes

^{*} Aided by the Ella Sachs Plotz Foundation.

¹ Thomsen, P., Altamirano, M., y Luco, J. V., Medicina (Buenos Aires), 1942, 3, 67.

² Rosenblueth, A., and Cannon, W. B., Am. J. Physiol., 1940, 130, 205.

do not return to normal simultaneously. The first to reappear is the response of the muscle to indirect stimulation at high frequencies, then the response to lower frequencies, then the sensitivity to curare, and lastly the post-tetanic potentiation.

The recuperation of normal functional phenomena as described above takes place in muscle in which the atrophic state persists, which suggests a lack of definite relationship between these disturbances and muscular atrophy.

A hypothesis was previously advanced1 that

the cause of disturbances resulting from tenotomy might be an alteration of the postural reflexes. The present study shows that the change is reversible, an observation in accord with the previous hypothesis. Of course there is also the possibility that the change in tension resulting in the muscle from the sectioning of its tendon may influence the neuromuscular junction directly.

Summary. The disturbance in neuroniuscular transmission caused by sectioning of the tendon disappears when the tendon is spontaneously re-established.

## 15199

Effect of Neostigmine (Prostigmin) and Physostigmine upon the Denervated Iris of the Cat.

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Medical School, Philadelphia, Pa.

It has been shown by Anderson^{1,2} that physostigmine constricts the pupil in normal cats but fails to do so after the ciliary ganglion has been removed and time permitted for degeneration of the post-ganglionic fibers. Similar studies have never been reported for neostigmine (prostigmin). In view of the differences known to exist between physostigmine and neostigmine (the latter being much more effective in relieving muscle weakness in myasthenia gravis) it is possible that neostigmine may have a direct action upon muscle in addition to its known anticholinesterase action. We decided to test this hypothesis so far as iris muscle is concerned.

Methods. Cats were anesthetized with sodium pentobarbital intravenously or intraperitoneally. The hair was shaved from the region of the left eye, the lids were sutured together and a skin incision was then made below and lateral to the orbit. A portion of the zygoma and lateral wall of the orbit was removed. The conjunctiva and Tenon's cap-

sule of the globe were opened and the external rectus muscle identified. The latter was retracted upward and the ciliary ganglion was exposed. If difficulty was encountered, the nerve from the inferior oblique muscle was traced back to its branch which leads to the ciliary ganglion. The ganglion was grasped by a fine hemostat, all its branches were severed, and the ganglion was removed. The wound was then flushed with penicillin solution and the incision was sutured. Since the operation abolished corneal sensitivity and secretion at least temporarily, the chief postoperative complication was desiccation and ulceration of the cornea; a number of cats had to be discarded for this reason.

In some cats the left superior cervical ganglion was also removed. This ganglion was distinguished from the vagus ganglion at the level of the carotid bifurcation and the former was extirpated completely.

Criteria of a successful ciliary ganglionectomy were: (a) pupillary dilation on the operated side, (b) failure of the iris to contract after exposure of the retina to strong light, (c) sensitivity to 0.5% pilocarpine and

¹ Anderson, H. K., J. Physiol., 1905, 33, 156.

² Anderson, H. K., J. Physiol., 1905, 33, 414.

TABLE I

			Effect	of	neostig	mine	bron	uide ervat	(5%) ed iris	insti mus	lled l	locally	into	eyes	with
	Ciliary	Superior Cervical					Day	rs						Week	ıs
Cat No.	ganglion- ectomy	ganglion- ectomy	1	2	3	4	6	7	8	9	10	14	3	4	10
1 2 3 4	× × ×							0	0	0	0				+++
6 7 8 10 11	× × × ×	×	++ ++				0	0							+++
12 13 14 15 16 17	× × × ×	× × × × ×	++++++++	++ +- ++	+ ++ + - 0 0 0	0			0				0		

0 = No response to 5% neostigmine bromine.

20% mecholyl on the operated side, and (d) failure of 2% physostigmine to constrict this pupil.

Pupillary diameters (horizontal) were measured with a millimeter rule, in the dimmest light compatible with accurate reading; this light was kept constant throughout the experiments. Five per cent neostigmine bromide was used throughout; 2 drops were instilled into the conjunctival sac of the eyes on both the normal and operated side.

Results. The effect of neostigmine upon the operated eyes is shown in Table I. In cats No. 1 to No. 8 the left ciliary ganglion had been removed and in cats No. 10 to No. 17 both the ciliary and superior cervical sympathetic ganglia had been extirpated. While complete data covering a period of 3 months are not available on any single cat, the results obtained in this series of 15 cats indicate:

- (1) In the first 1 to 3 days postoperatively the denervated iris is unusually sensitive to neostigmine, the denervated side constricting much more fully than the normal side (Table I).
- (2) After the 2nd or 3rd day following ciliary ganglionectomy the iris becomes insensitive to neostigmine whether the ciliary alone or this plus the superior cervical sympathetic

ganglia had been removed.

(3) By the 10th week (and possibly earlier) the "denervated" side had recovered its ability to respond to neostigmine.

We made no attempt to determine the earliest time at which pilocarpine sensitivity appeared following ciliary ganglionectomy but when this drug was instilled for the first time 7, 8, 9, or 14 days postoperatively, sensitivity already existed. Reactions to physostigmine were similar to those to neostigmine in every instance in which both were measured.

Comment. After an initial period of sensitivity to neostigmine (which was probably due to traumatic injury to the postganglionic fibers) the denervated pupil failed to respond to 5% neostigmine. Later (2½ months) neostigmine again produced pupillary constriction; this was probably due to regeneration of postganglionic fibers from ganglion cells still present in the distal nerve trunk.

The failure of the denervated pupil to react to neostigmine proves that in the concentration and by the route employed, neostigmine has no direct action upon the circular muscle of the iris. Even when the dilator sympathetic tone (which might oppose a weak constrictor effect of neostigmine directly on the iris) was removed, neostigmine was still without effect. The action of physostigmine

^{+ =} Normal response to 5% neostigmine bromine. ++ = Increased response to 5% neostigmine bromine.

was similar in all respects to that of neostigmine. The studies on physostigmine confirm those carried out by Anderson¹ though the latter have been erroneously interpreted by Meyer and Gottlieb³ as evidence of a direct action of physostigmine on the iris.

The action of physostigmine and neostigmine therefore is one wholly due to inactivation of cholinesterase, and consequent preservation of stimulant quantities of acetylcholine. For the production of acetylcholine, some or all of the ganglion cells must be intact or there must exist some local stimulation of postganglionic fibers.

Conclusion. Neostigmine (prostigmin) does not stimulate the denervated iris muscle of the cat. It causes miosis only when some or all of the ciliary (parasympathetic) ganglion cells are intact or when the postganglionic fibers are stimulated.

## 15200

# Effect of Intermittent Exposure to Simulated High Altitude in Erythropoiesis in the Guinea Pig.*

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Investigations on the effects of intermittent exposure to high altitudes have been pursued only since 1938 when Armstrong and Heim¹ subjected rabbits to a simulated altitude of 18,000 feet, 4 hours daily for 5 weeks. Their animals failed to show an increase in red blood corpuscles in adaptation to the lowered oxygen tension. They also found2 that young men exposed intermittently to an altitude of 12,000 feet showed evidence of only slight acclimatization. On the other hand, Stickney and his collaborators3,4 found that dogs subjected discontinuously to an altitude of 18,000 feet for 16 weeks did exhibit an increase in the amount of hemoglobin and number of red blood corpuscles.

Material and Methods. Fifty-eight young adult male guinea pigs were used. Twenty-six of these served as controls; the remainder were subjected to discontinuous exposures to air at lowered barometric pressure for 6 hours daily, except Sunday. In the first experiment the simulated altitude was 23,000 feet (307 mm Hg pressure); in the second experiment, 30,000 feet (225 mm Hg pressure).

The conditions of the concurrent neurological experiment required that 3 to 6 animals be sacrificed periodically as the whole series accumulated exposure time. In the first experiment, groups were sacrificed after 100, 200, 300, and 500 hours of exposure. In the

³ Meyer, H. H., and Gottlieb, R., Die Experimentelle Pharmakologie, 8th edition, 1933, Berlin, page 189.

The purpose of this report is to present further data from a different species, the guinea pig. At first our study was incidental to an investigation of the effect of exposure to simulated high altitude on the structure of the brain⁵ and we made hemoglobin determinations only. Later the blood study was expanded, though done in conjunction with the neurological study; the same animals were used in both problems.

^{*} Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

¹ Armstrong, H. G., and Heim, J. W., J. Aviat. Med., 1938, 9, 92.

² Armstrong, H. G., Principles and Practice of Aviation Medicine, Williams and Wilkins Co., Baltimore, 1943.

³ Stickney, J. C., and Van Lierc, E. J., J. Aviat. Med., 1942, 13, 170.

⁴ Stickney, J. C., Northrup, D. W., and Van Liere, E. J., PROC. Soc. EXP. BIOL. AND MED., 1943, 54, 151.

⁵ Windle, W. F., and Jensen, A. V., J. Aviat. Med., 1945, in press.

TABLE I.

Changes in Mean Hemoglobin Coutent of the Blood During Discoutinuous Exposure to Simulated High Altitude.

Group	No. animals	Altitude feet × 1000	Exposure time, hr	Hb. g/100 cc	σ
Controls	17			14.4	± 0.89
A	3	23	100	19.5	$\pm 0.40$
В	3	30	100	18.3	$\pm 1.53$
C	4	23	204	25.5	$\pm 2.38$
D	4	23	300	26.5	$\pm 1.00$
E	4	23	500	25.2	$\pm 0.97$
F	6	{ 23	100 }	24.4	$\pm 1.19$
		í 30	100 (		
G	4	`30	200	24.5	$\pm 1.91$
$\mathbf{H}$	4	30	250	22.9	$\pm 1.17$

second experiment groups were sacrificed at 100, 200, and 250 hours. Six other animals received 100 hours at 23,000 feet followed by 100 hours at 30,000 feet.

Blood studies were made on each animal just before it was killed 17 to 24 hours after the last exposure to reduced oxygen tension. Normal controls for each group were sacrificed at the same time and in the same manner. All studies were made on cardiac blood which was placed in a bottle containing an ammonium potassium oxalate mixture. The hemoglobin was determined by the Hellige-Sahli method, with an instrument calibrated by the oxygen capacity technic. The erythrocyte counts were performed with U.S. Bureau of Standards pipettes and chambers. Hematocrit determinations were made in the Wintrobe tube. Centrifugation was carried out at 2800 revolutions per minute for 30 minutes. After vital staining reticulocytes were estimated on unstained dried smears.

Results. The data from both experiments are presented in two tables. Table I shows

the average hemoglobin values for each group of experimental animals and 17 controls. The table indicates that following 100 hours of exposure to 23,000 feet (A) or 30,000 feet (B) the amount of hemoglobin was essentially the same, about 19 g per 100 cc. Within the limits of experimental error both values were equal. After 200 hours exposure to either altitude, (C and G) there was no further increase of significance. Even after 500 hours (E) the amount of hemoglobin did not increase over that found after 200 hours, about 25 g per 100 cc. After 100 hours at either altitude the animals were about one-half acclimatized: after 200 hours at either altitude, or a combination of the two altitudes (F) the animals were fully acclimatized.

In Table II all data on the blood picture are summarized. The figures represent average values for each group. Each group is designated by a letter corresponding to those in Table I. Average values for 9 normal controls are presented for purposes of comparison. The groups have been placed in the table, so far as

TABLE II.

Average Changes in Blood Values Obtained During Discontinuous Exposure to Simulated High Altitude.

Group	No. animals	Altitude, feet × 1000	Exposure time, hr	R.B.C., mil.	Hb., g/100 cc	Hemat., %	Retic., %	Spleen wt., g/kg
Control	9			5.07	13.7	43	0,9	1.67
${f B}$	3	30	100	6.51	18.3	60	1.3	2.39
$\mathbf{F}$	6	{ 23	100 }					
		) 30	100 (	7.70	24.4	76	1.1	3.26*
G	4	`30	200	9.04	24.5	79	1.4	2.09
$\mathbf{H}$	4	30	250	8.15	22.9	72	3.2	2.25
$\mathbf{E}$	.1	23	500	8.19	25.2	72	2.5	3.49

^{*} Avg of 4 animals.

possible, in the order of severity of exposure to lowered oxygen tension.

An increase in number of red-blood corpuscles and in hematocrit and hemoglobin values proceeds up to 200 hours of exposure time after which there is a leveling off. Neither 250 hours at 30,000 feet or 500 hours at 23,000 feet caused an increase over the values obtained for 200 hours at 30,000 feet. A greater degree of reticulocytosis was obtained following the more severe experimental conditions (H and E). Enlargement of the spleen occurred.

The maximum number of red blood cells encountered in any animal was 10.04 million. This animal had a hemoglobin value of 26.0 g per 100 cc and an hematocrit of 87%. The animal belonged in Group G. Several other animals from this and other groups also showed increases of almost 100% over the mean control values for hemoglobin, red corpuscles and hematocrit. These values are similar to maximal values seen in patients with polycythemia vera.

The results obtained in this Comment. study demonstrate that an extraordinary degree of polycythemia may be produced by intermittent exposure to simulated high altitude. Among the 32 experimental animals subjected to varied grades of severity of experimental conditions there was no single animal in which a notable increase in amount of hemoglobin and red blood corpuscles did not occur. It should be borne in mind that the animals were exposed to low oxygen tensions for only 6 hours a day and 6 days a week. The degree of anoxia used was much greater than man can tolerate; e.g., man cannot ascend to altitudes over 18,000 feet for more than a few minutes at a time without

experiencing impairment of psychological and physiological functions, unless he is breathing air_enriched_with-oxygen.-Armstrong2_statesthat at an altitude of 25,000 feet death may occur in man at any time after 20 to 30 minutes. At 30,000 feet the guinea pigs behaved lethargically and exhibited a moderate hyperpnea when active, but they were otherwise apparently normal. So long as they remained quietly huddled in their usual position no physiological distress was observed. They could even make a small amount of movement without deleterious effects. With greater exertion, however, they fell upon their sides and remained there with legs outstretched and apparently in a coma for 30 to 60 seconds, afterwards righting themselves and remaining quiet. It appears, therefore, that the critical altitude for the guinea pig lies at about 30,000 feet. Its "ceiling" is something more than 5,000 feet higher than that of man.

The enlargement of the spleens in these animals is probably related to the polycythemia. Our results on this point support the continuous exposure experiments of Van Liere on guinea pigs, and the experiments of Van Liere and Stickney⁷ on dogs.

Conclusions. Guinea pigs exposed to simulated altitudes of 23,000 and 30,000 feet 6 hours a day and 6 days per week became fully acclimatized after 200 hours, as shown by maximal increase in the values for red corpuscles, hemoglobin, and hematocrit. Reticulocytosis and enlargement of the spleen were also demonstrated.

⁶ Van Liere, E. J., Am. J. Physiol., 1936, 116,

⁷ Van Liere, E. J., and Stickney, J. C., J. Aviat. Med., 1943, 14, 194.

### 15201

## Failure of Intestinal Extracts to Prevent Chick Gizzard Erosions.

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Swift and Co., Chicago, 111.

It has recently been shown¹ that extracts of the upper intestine of hogs are effective when administered orally in preventing jejunal ulcer in dogs subjected to the Mann-Williamson operation. This investigation was undertaken to determine whether these intestinal extracts contain any of the chick anti-gizzard erosion factor.

Methods. The intestinal extract used in this study was material which had been shown to be effective in preventing ulcer in Mann-Williamson dogs. Its preparation consisted essentially of extracting the upper small intestine of hogs with 0.4% hydrochloric acid, concentrating this extract in vacuo, and drying of the concentrate from the frozen state. The dried extract was fed as 5% of a ration known to produce gizzard erosion in chicks.

One-day-old White Rock chicks were distributed at random into two groups of 20 chicks each. Group 1 was fed the control ration, and Group 2 was fed the test ration, consisting of the control ration supplemented with 5% of the dried extract. The basal ration, modified slightly from one used by Miller and Hammond,² had the following

composition: ground yellow corn 39.9%, ground whole wheat 21.0%, dried skim milk 10.0%, meat and bone scraps 10.0%, soybean oil meal 10.0%, alfalfa leaf meal 3.0%, dried brewer's yeast 2.0%, steamed bonemeal 3.0%, salt 1.0%, and vitamin A and D feeding oil (3000 USP units vitamin A and 400 AOAc chick units vitamin D per gram) 0.1%. The chicks were banded and weighed individually at the start of the experiment, at 2 weeks of age, and at 4 weeks of age when the experiment was concluded. The average weights of the birds at 4 weeks of age were for Group 1, 226 g, and for Group 2, 238 g. The chicks were killed by decapitation and examined for gizzard erosions. The incidence of erosions in Group 1 was found to be 75%, and in Group 2, 67%. The severity of the lesions was about the same in the two groups.

A similar experiment with salt precipitated material (the "A" precipitate of Greengard and Ivy³) also gave negative results when fed at a 5% level in this ration.

Summary. The intestinal extract used in this study apparently was not effective in preventing gizzard erosion in chicks.

¹ Ivy, A. C., Fed. Proc., 1945, 4, 222.

² Miller, D., and Hammond, J. C., Poultry Sci., 1942, 21, 317.

³ Greengard, H., and Ivy, A. C., Am. J. Physiol., 1938, 124, 427.

# Carbon Tetrachloride Injury of the Liver. The Protective Action of Certain Compounds.*

Alexander Brunschwig, Charles Johnson, and Sabra Nichols. From the Department of Surgery, University of Chicago.

In a previous communication results of experiments in dogs were reported showing that sodium thioglycollate conferred the same order of protection of the liver against acute chloroform injury as did protein and methionine. These studies were interpreted as showing the importance of the -SH group in the protective mechanisms of the hepatic cell against one form of acute injury. The criterion for hepatic damage was the bromsulphalein excretion-a physiologic test. Histologic study of the livers was not carried out because of the nature of the experiments.

This report is concerned with a study of the protective action of certain -SH and other compounds against acute hepatic injury produced by carbon tetrachloride in the rat. In this study histologic criteria were employed. The types of injury produced by carbon tetrachloride and chloroform respectively are not necessarily closely related phenomena.

Injection of .1 cc of carbon tetrachloride subcutaneously into rats weighing 150 g results in 24 hours in characteristic hepatic lesions.2 There are many foci of enlarged hepatic cells with clear vacuolated cytoplasm; the nuclei are slightly reduced in size. In the central portions of such foci, hepatic cells are shrunken, and their cytoplasm is dense and eosinophilic. Associated with these lesions there is a varying degree of round cell infiltration. Most of the foci appear about the central lobular vein; some are in the periphery of the lobules. These lesions are ephemeral since 2 weeks after a single injection they are no longer present and the liver appears normal.

The vacuolation of the hepatic cells is not closely associated with the accumulation of fat in the liver since staining with Sudan III reveals no fat in the enlarged cells. latter are the result of acute edema.

Fifteen rats received injections of .1 cc of carbon tetrachloride subcutaneously and a maximal extent of the lesions described above was observed in histologic sections of the liver taken at necropsy 24 hours later. In 2 animals the lesions were of limited size. These findings indicate that in exceptional instances there is an unusual degree of resistance to the noxious effects of carbon tetrachloride.

A series of observations were then carried out in which various agents were injected intraperitoneally immediately following the subcutaneous injection of carbon tetrachloride. The animals were killed 24 hours later and the livers removed for histologic study. Failure of development of the lesions or an appreciable reduction in their number and size was interpreted to indicate that the agent injected intraperitoneally conferred a degree of protection against the acute injury by carbon tetrachloride (Fig. 1).

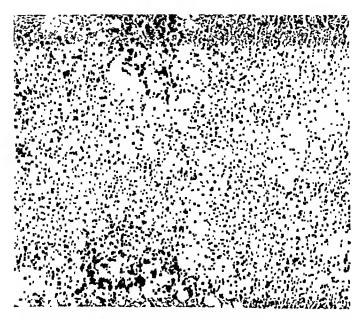
The results are summarized in Table I. Since the occasional animal appears to be resistant to the effects of carbon tetrachloride a definite protective action was ascribed to an injected agent only if at least approximately 40% of the animals tested showed lesions of limited extent or no appreciable lesions. The table reveals that a very marked protection was afforded by sodium thioglycollate; considerable protection was also obtained with sodium glycollate. Glutathione afforded appreciable protection. Sodium thiomalate, sodium malate, and cystein afforded definite protection but less marked than the previously

^{*}This study was conducted with aid of the Charles and Mary F. S. Worcester Fund, University of Chicago.

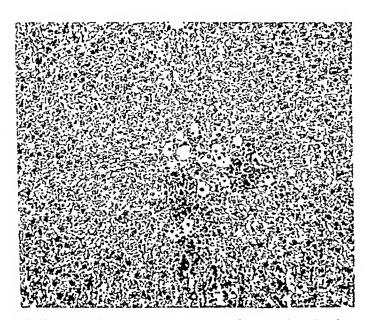
¹ Brunschwig, A., Nichols, S., and Bigelow, R. R., Arch. Path., 1945, 40, 81.

² Cameron, G. R., and Karunaratne, W. A. E., J. Path. and Bact., 1936, 42, 1.

Fig. 1.



A. Typical maximal lesions in liver of rat 24 hours after injection of .1 ec carbon tetrachloride subcutaneously. Foci of shrunken cells surrounded by markedly swollen cells.



B. "Moderate" lesion observed 24 hours after injection of earbon tetrachloride and sodium glycollate.

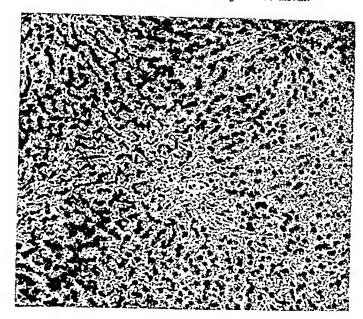


Fig. 1.
C. Minimal lesions (slight edema of some cells) after injection of carbon tetrachloride and glutathione.

mentioned compounds. Aspartic acid, glutamic acid (both as sodium salt), sodium acetate, methionine, choline alone, and choline plus cystine did not afford significant protection.

A protective action against injury by carbon tetrachloride does not necessarily depend upon the presence of -SH in the agent tested, as was the case in studies previously reported and mentioned above concerning the protective

TABLE I.
Summary of Results of Experiments on Protective Action of Various Compounds Against Hepatic Injury by Carbon Tetrachloride.

				ree of ection. f rats	1,		
Agent injected	No. rats	Amt. (150 g rat)	Snlphur, 150 g rat	No. showing maximal lesion	Mod- erate lesions	Slight or no lesions	% with moderate or no lesions
CCl	15	.1 ce		13	1	1	13
CCl. + Sod. thioglycollate	13	17.8 mg	$6.2 \mathrm{\ mg}$	0	2	11	100
CCl ₄ + Sod. glycollate	13	14.8		2	1	11	84
CCl. + Disod. thiomalate	19	29.1 ''	6.2 mg	11	4	4	42
CCl. + Disod. malate	14	26 ''		8	3	3	42
CCl. + Sod. thiosulph.	10	25 "	$6.2 \mathrm{mg}$	7	1	2	30
CCl. + Disodaspartate	5	55.2 ''		4	1	0	20
CCl4 + Disod. glutamate	5	60 ''	-~	4	1	0	20
CCl4 + methionine	19	$60 \cdot 120 \text{ mg}$	12.4-24.8	18	0	1	5.3
CCl ₄ + choline	3	40 mg		3	0	0	0
CCl4 + choline and cystine	. 8	30 '' choline 25 '' cystine	6.2	8	0	0	0
	10	(per os) 34.5 mg	6.2	8	9	3	38
CCl ₄ + cystein	13	60 ''	6.2	4	$\tilde{2}$	4	60
CCl ₄ + glutathione CCl ₄ + Sod. acetate	10 9	116 "		8	<u> </u>	i	

^{*} All rats received .1 ce carbon tetrachloride per 150 g weight in addition to other injections.

action of protein and other agents against chloroform injury of the liver in dogs. The intracellular disturbances in the liver occasioned by chloroform and carbon tetrachloride intoxication respectively probably represent different phenomena. Evidence for this is the fact that a protective action was observed for both sodium thioglycollate and sodium glycollate against carbon tetrachloride injury in rats whereas in experiments in dogs with injury, sodium thioglycollate chloroform afforded protection but none was observed with sodium glycollate, indeed the liver appeared to be injured by this substance (unpublished experiments). Conversely, methionine afforded protection against chloroform injury in dogs but did not afford protection against carbon tetrachloride injury in rats.

Summary. Several agents were tested for protective action against carbon tetrachloride

injury of the liver in rats. Marked protection was obtained with sodium thioglycollate, appreciable protection with glutathione, moderate protection with sodium thiomalate, and slight protection with cystein—all sulphydryl compounds. Sodium glycollate and sodium malate afforded protection comparable to their sulphydryl homologues. Methionine, and choline plus cystine did not afford protection. Other non-sulphydryl compounds, i.e., sodium thiosulphate, aspartic acid, glutamic acid, choline, and sodium acetate afforded no protection.

A protective action against carbon tetrachloride injury of the liver in rats is not as closely related to the presence of -SH in the test agent as previously was observed for the protective action of certain compounds against hepatic injury by chloroform in the dog.

#### 15203

## Blood Pressure Changes During Electronarcosis.

A, VAN HARREVELD AND W. B. DANDLIKER.

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The blood pressure during the first phases of electronarcosis¹ shows large and complicated variations, mainly caused by the simultaneous stimulation of the sympathetic system and the vagus nerves.¹⁻⁵ The possibility that humoral mechanisms, other than those at the autonomic nerve endings, are active in producing these pressure changes, is here considered.

Method. Electronarcosis was produced by applying a 60-cycle alternating current to the brain through electrodes placed on both sides of the head directly behind the eyes. As usual, a relatively high current, in most cases 150 ma, was applied for the first 30 seconds, and then decreased to about 30 ma for the rest of the electronarcosis. Rabbits were used exclusively.

Recording mercury manometers were used for the registration of the blood pressure. Fastusol⁶ was used to prevent blood clotting during the recording as well as in crossed circulation experiments.

The Blood Pressure Changes During Electronarcosis. On starting the high initial current, the blood pressure drops due to vagus inhibition, causing arrest of the heart. After

¹ Frostig, J. P., van Harreveld, A., Reznick, S., Tyler, D. B., and Wiersma, C. A. G., Arch. Neurol. Psych., 1944, 51, 232.

² Bikeles, G., and Zbyszewski, L., Arch. f. d. ges. Physiol., 1920, 182, 157.

³ Ivy, A. C., and Barry, F. S., Am. J. Physiol., 1932, 99, 298.

⁴Roos, J., and Koopmans, S., Vet. J., 1934, 90, 232.

⁵ van Harreveld, A., and Kok, D. J., Arch. Néerl. de Physiol., 1934, 19, 24.

⁶ Modell, W., Science, 1939, 89, 349.

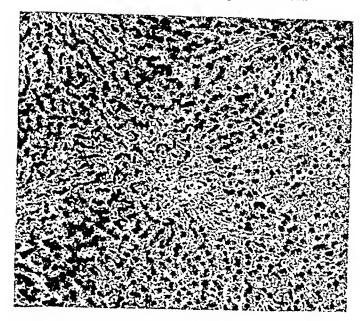


Fig. 1.
C. Minimal lesions (slight edema of some cells) after injection of carbon tetrachloride and glutathione.

mentioned compounds. Aspartic acid, glutamic acid (both as sodium salt), sodium acetate, methionine, choline alone, and choline plus cystine did not afford significant protection.

A protective action against injury by carbon tetrachloride does not necessarily depend upon the presence of -SH in the agent tested, as was the case in studies previously reported and mentioned above concerning the protective

TABLE I.
Summary of Results of Experiments on Protective Action of Various Compounds Against Hepatic Injury
by Carbon Tetrachloride.

			Degree of Protection No. of rats						
Agent injected	No. rats	Amt. (150 g rat)	Sulphur, 150 g rat	No. showing maximal lesion		Slight or no lesions	% with moderate or no lesions		
CCl ₄	15	.1 ce		13	1	1	13		
CCl4" + Sod. thioglycollate	13	17.8 mg	6.2 mg	0	2	11	100		
CCl ₄ + Sod. glycollate	13	14.8		3	1	11	84		
CCl ₁ + Disod. thiomalate	19	29.1 **	6.2 mg	11	4	4	42		
CCl ₄ + Disod. malate	14	26 ''		8	3	3	42		
CCl ₄ + Sod. thiosulph.	10	25 ''	$6.2 \mathrm{\ mg}$	7	1	2	30		
CCl ₄ + Disodaspartate	5	35.2 ''		4	1	0	20		
CCl ₄ + Disod. glutamate	5	60 ,,		4	1	0	20		
CCl ₄ + methionine	19	60-120 mg	12,4-24.8	18	0	1	5.3		
CCl ₄ + choline	3	40 mg		3	0	0	0		
CCl ₄ + choline and eystine	8	30 '' elioline 25 '' eystine (per os)	6,2	8	0	0	0		
	13	34.5 mg	6.2	8	2	3	38		
CCl ₄ + eystein	10	60 ,	6,2	4	2	4	60		
CCl ₄ + glutathioue CCl ₄ + Sod. acetate	9	116 "		8	0	1			

All rats received .1 ee earbon tetrachloride per 150 g weight in addition to other injections.

mum within the first 30 seconds of high initial current (Fig. 1). With vagi severed or intact the blood pressure drops, sometimes considerably below the prenarcotic value, after the current is decreased to the 30 ma maintenance level. In the further course of electronarcosis a second increase of blood pressure usually develops which reaches its maximum 2 to 3 minutes after the start. Neither the respiratory arrest (if not too prolonged) nor the strong convulsions present during the first phases of electronarcosis have a pronounced effect on the course of the blood pressure changes, since continuous artificial respiration applied to the completely curarised* animal does not alter materially these changes.

The sudden drop in blood pressure is clearly related to the change from high to maintenance current, since it is delayed when the initial high current is prolonged. If the high current is maintained for two minutes, for instance, the pressure, after reaching a maximum within the first 30 seconds, decreases slowly until the current is cut, when it drops quickly below the prenarcotic value (Fig. 2). (This experiment was performed in the curarised animal under artificial respiration, since the continued high current prevents the return of spontaneous respiration.)

Humoral Mechanisms in the Blood Pressure Changes During Electronarcosis. The release during electronarcosis of vasoactive substances has been studied in crossed circulation experiments. A symmetrical double carotid-jugular anastomosis was made between electronarcotised and test rabbits. The blood streams were so regulated that the blood pressure in each animal remained constant, indicating an equal flow in both streams. The flow into the test rabbit, which passed through a flow meter, was in most experiments of the order of 100 cc/minute, and was kept constant by a screw clamp regulator, despite blood pressure changes in the electronarcotised animal. In this way blood pressure changes in the test rabbit from mere changes in its blood volume were prevented. In both animals the vagus nerves were severed bilaterally.

Fig. 3 I is an example of such an experiment. About 8 seconds after the start of electronarcosis the blood pressure of the test animal starts to rise, reaching a maximum in about 30 seconds, after which the pressure slowly returns to its original level. The injection of 10⁻³ g adrenaline in the jugular vein of the animal previously subjected to electronarcosis, causes the blood pressure increases of Fig. 3 II.

The rise in pressure in the test rabbit is largest during the first current application; on repetition at intervals of 10 minutes it soon vanishes, though the pressure changes in the electronarcotised animal remain essentially alike.

Ellis and Wiersma7 have shown that electronarcosis increases the release of tropic hormones of the pituitary gland. The blood pressure rise in the test rabbit might therefore be due to pitressin. This possibility was investigated by recording the blood pressure in animals in which the nervous connections of the head with the body were severed as completely as possible. A high spinal transection (C 5) was performed 2 days before the experiment, and vagus and phrenic nervest were severed shortly before applying electronarcosis. In such animals the effect of electronarcosis on the blood pressure was minor, indicating that a hypophyseal effect, if present at all, is a negligible factor in the pressure rise.

The strong excitation of the sympathetic system during electronarcosis probably causes a release of adrenaline (and perhaps of sympathin) in the general circulation; and this substance may cause the blood pressure rise in the test animal in the crossed circulation experiments.

Conclusions. A comparison of the blood pressure rises in the electronarcotised and test animals during electronarcosis and after the injection of 10⁻³ g adrenaline (Fig. 3 I and II) shows that the rise during electronarcosis

^{*} Intocostrin (E. R. Squibb and Sons, New York) was used.

⁷ Ellis, C. H., and Wiersma, C. A. G., Proc. Soc. Exp. Biol. and Med., 1945, 58, 160.

t The circulation in the high spinal rabbit is so precarious that respiratory arrest often caused a considerable drop in blood pressure, probably by impairing the veuous return to the heart. Artificial respiration, after severing the phrenic nerves, was therefore used.

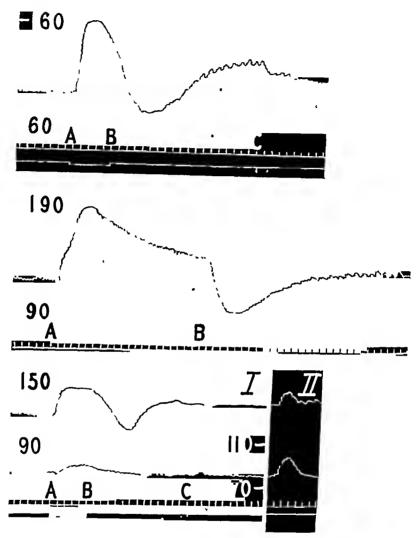


Fig 1 Rabbit, 50 mg nembutal, 1 cc intocostrin, vagus nerves cut At "A" a current of 150 ma is applied for 30 sec This is decreased to 30 ma at "B" and the current is cut at "C" Time 6 sec Blood pressure in imm mercury.

Fig 2 Preparation of the animal as in Fig 1 At "A" a current of 150 ma is applied, which is cut after 2 minutes at "B"

Fig 3, I and II Crossed circulation experiment, 50 mg nembutal was ad ministered to both animals, the electionarcotised rabbit obtained 1 cc intocostrin (injected before the crossed circulation was established). Blood flow 110 cc/min. The upper blood pressure accord is from the electionarcotised animal, the lower from the test labbit. At "A" a current of 150 ma was applied, which was decreased to 30 ma at "B," and cut at "C". Fig 3 II shows the effect of the injection of 10-5 gram adrenaline into the jugular vein of the rabbit which was electronarcotised before

a few seconds, the heart escapes and the blood pressure rises considerably above the prenarcotic value because of the concomitant vasoconstriction induced by sympathetic stim-

#### ulation 1

After severing both vagi the blood pressure begins to rise almost immediately after the current is started, usually reaching a maxi-

TABLE I.

Minimum Fungistatic Concentration of Fractions Preventing Growth of Microsporon audomini in Sabouraud's Medium.

Total fat Free fatty acid	0.6 % 0.05 %	Free fatty acid Steam distillate	0.05 % 0.005 %	Remaiuder	$ \begin{array}{ccc} 5.0 & \% \\ < 0.4 & \% \end{array} $
Steam distillate	0.005%	First portion of frac- tionate distillation	0.0005%	,,	0.005%

cated that the material was not pure. The C:H relation roughly corresponded with that of aliphatic fatty acids, but a strong purple color with ferric chloride indicated the presence of a phenolic compound. In each sample the neutral equivalent was higher than the molecular weight (camphor method), an indication that the impurities were not acids. Strong unsaturation reaction was observed. No sulfur and only traces of nitrogen were present.

In a third experiment we succeeded to separate the phenolic impurity by freezing the distillate. Upon warming it slowly a large oil droplet could be easily pipetted off from the top of the still hard frozen phenol. The oily drop gave no color reaction with ferric chloride and no unsaturation reaction. The phenolic impurity, causing these reactions in earlier samples, had no fungistatic action.

The most active fatty acid fractions in our experiments inhibited the growth of M. audouini between 0.0002 and 0.001%. This is in the range of the fungistatic activity of the  $C_7$ ,  $C_9$ , and  $C_{11}$  straight chain aliphatic fatty acids on the same fungus.

At present the fat from 45 kg of hair is being fractionated. This amount possibly will be sufficient for separation and final chemical analysis of the fatty acids present in the active distillate.

Summary. Hair fat of adults contains free saturated aliphatic fatty acids which inhibit the growth of M. audouini in concentrations of 0.0002 to 0.001%. Preliminary chemical analyses of the still impure fractions, molecular weight estimations, and the range of fungistatic activity indicate that the fraction contains saturated aliphatic fatty acids between  $C_7$  and  $C_{11}$ .

TABLE II.
Quantitative Chemical Analysis of Fractional Distillation Products.

			Fraction II July, 1945	
H C	10.54%	10.40%	11.34%	10.30%
•	68.57%	69.52%	73.25%	65.79%
Neutral equivalent	176	198	223	159
Molecular weight	145	154	185	133
Test for phenolic group Purple color	distinct	distinct	none	попе
Test for unsaturation (KMnO ₄ ) decolorization	very rapidly	rapidly	slowly	very slowly
Test for unsaturation (Br ₂ ) decolorization	slowly	slowly	slowly	none

^{*} Contains phenolic impurity.
† Phenolic impurity removed.

(after elimination of the vagus effect) can be explained only for a small part by hormonal mechanisms. It must be mainly a nervous phenomenon.

The considerable drop in pressure, which occurs when the high initial current is reduced, may be due to a period of decreased responsiveness of the sympathetic system after the strong stimulation. Normal stimuli and those set up by the lower maintenance current would

then be unable to maintain the high or even the normal blood pressure. This is supported by the observation that, when electronaccosis is repeated at intervals of 10 minutes, this drop tends to become more marked.

The second maximum in blood pressure (Fig. 1) may be caused by impulses produced in the sympathetic by the smaller maintenance current, after this system has recovered from the effects of the high initial current.

## 15204 P

## Fungistatic Action of Hair Fat on Microsporon audouini.*

Stephen Rothman, Adelaide M. Smiljanic, and Arthur L. Shapiro.

From the Section of Dermatology, Department of Medicine, University of Chicago, Chicago, Ill.

In the last few years ringworm of the scalp caused by Microsporon audouini has been endemic among school children in the major cities of the United States. This infection spontaneously with oncoming adolescence, and the scalp hair of adults is completely immune to the invasion of the fungus. The non-hairy skin remains susceptible in adults.1 It has been obvious that the change in susceptibility during adolescence is due to the sudden development of sebaceous glands of the scalp under the influence of sex hormones, and to the associated changes in the chemical composition of the sebum. In this laboratory it was attempted to trace these changes in order to find the explanation for the immunity of adults' hair.

Experimental. Hair of adults was pooled from barber shops and extracted with hot ether. The hair fat was added to Sabouraud's agar medium in graded concentrations. The agar tubes were then inoculated with a standard culture of M. audouini. It was found that hair fat had an inhibitory action on the growth of the fungus, and that the minimum concentration preventing growth was 0.5%. Examining the hair of medical students who did not use any hair tonic or pomade in a

one-week period between washing and cutting the hair, we ascertained that the fungistatic effect was not caused by extraneous material. Fat from children's pooled hair had ½ the fungistatic action of adults' hair fat.

Adults' hair fat was fractionated and the activity of the fractions was assayed on M. audouini cultures (Table I). By treating the fat with cold 1% NaOH the free fatty acid fraction was split off. This fraction contained the whole fungistatic principle whereas the remainder, containing neutral fats, cholesterol, and other unsaponifiable material, was inactive. Subsequently, steam distillation of the free fatty acid fraction was carried out, and the active principle was recovered from the steam distillate. The final step was fractionation of the steam distillate. The first fraction distilling over between 90 and 105°C at 1 to 2 mm Hg pressure contained most of the fungistatic material.

This active fraction was isolated in 2 separate experiments. In both cases 12 kg of hair was used. The yield of active fraction was 20.3 mg and 35.5 mg respectively. A second fraction (55.5 mg) distilling around 110°C was isolated once and had similar fungistatic power to the lower boiling fraction. Microanalysis of these samples (Table II) indi-

^{*} Aided by a grant from Wallace & Tiernan Products, Inc., Belleville, N.J.

¹ Rothmar S., Hygeia, 1945, 23, 436.

[†] Microanalyses were performed by Dr. T. S. Ma, Department of Chemistry.

animals were totally adrenalectomized, given 1% salt solution and kept on the deficient diet. Four to 6 days after adrenalectomy, the hair follicles in the 6 surviving animals became active as shown by the pigmentation through the skin and 12 to 14 days after the operation well pigmented hair began appearing externally on the 4 surviving animals. Hair failed to regrow on the 2 rats which were not adrenalectomized. Thus hair better in quality and darker in pigmentation resulted in all of the adrenalectomized animals.

After the hair had been removed in twenty 22-day-old rats, the adrenals were transplanted via the dorsal approach into the kidneys as in previous experiments.⁸ The animals were kept on the deficient diet and 1% solution of sodium chloride served as drinking water for 5 or 6 days after the operation. Ten unoperated littermates with the hair removed from their backs were kept in the same cages. Sparse and dull gray hair appeared on the 18 surviving operated rats and the 10 unoperated between the ages of 45 and 60 days. Rats in which the adrenals were transplanted to the

kidneys were sacrificed after the appearance of the hair. Histological sections of the kidneys showed the persistence of cortical material of the adrenal transplant and the absence of the medullary portion. The well pigmented hair of good quality following total adrenalectomy in the previous experiments can not therefore be due to the absence of the medullary portion of the adrenal but must be related to the absence of the cortex of the adrenal.

Summary. Graying of the hair results in black rats fed a vitamin B-complex-free diet, supplemented with thiamine, riboflavin, and pyridoxine. New hair growing in after the ablation of both adrenal glands in rats kept on such a deficient diet is again well pigmented. To determine whether the increased pigmentation was due to the lack of the medullary or cortical portion of the adrenal, the adrenals were transplanted into the kidneys. The medullary portion of the adrenal degenerated, the cortex persisted, and poorly pigmented hair grew on the animals. Thus the lack of the cortex was related to the increased pigmentation following adrenalectomy.

#### 15205

## Effects of Adrenalectomy on Pigmentation of Hair in Rats Fed a Deficient Diet.

#### EARL O. BUTCHER.

From the Department of Anatomy, College of Dentistry, New York University.

It has been reported by Ralli and Graef¹ that graying of the hair in black rats will result from feeding a vitamin B-complex-free diet, supplemented with thiamine, riboflavin. and pyridoxine, but that new hair growing in after the ablation of both adrenal glands in rats kept on such a diet will again be well pigmented. No attempt was made by them to determine whether the pigmentation was due to the absence of the medulla or the cortex of the adrenal. Growth of the hair was also accelerated following adrenalectomy, confirming my earlier work.2 More recently Spoor and Ralli³ presented some evidence that the adrenal cortex was involved in the metabolism of the melanin. They observed that adrenalectomized animals fed the deficient diet and treated with desoxycorticosterone acetate did not become pigmented to the same degree as did the adrenalectomized controls.

The present investigation has been undertaken on rats fed a vitamin B-complex-free diet, supplemented with thiamine, riboflavin, and pyridoxine, to learn which part of the adrenal is related to the pigmentation following adrenalectomy.

Procedure and Results. In the present experiments hooded rats of the Long-Evans strain were used. They were kept on the deficient diet which consisted of casein 18%, sucrose 68%, vegetable oil 10% and salt mixture 4%, to which was added daily about 20  $\mu$ g of thiamin chloride, 20  $\mu$ g of pyridoxine and 60  $\mu$ g of riboflavin. Each animal also received one drop of halibut liver oil once a week during the period of the experiments. It has been well established^{1,4,5,6,7} that rats maintained on this diet exhibit graying of the hair.

The hair was removed from the backs of 12 rats with sodium sulphide when they were 22 days old and they were kept on the deficient diet. Sparse and dull gray hair appeared on

5 of the rats between the 45th and 50th days of life and on 4 of them between the 50th and 55th days. Very little hair regrew on the other 3 rats. Six litter-mates from which the hair was removed on the 22nd day and which were kept on a non-deficient diet (Purina dog chow) produced deeply pigmented hair usually on the 36th day of life.

The hair was removed from the backs of 20 other 24-day-old rats and they were completely adrenalectomized via the dorsal approach.8 Ten littermates in which a mock adrenalectomy was performed served as controls. All rats were fed the deficient diet and given 1% sodium chloride as drinking water. By the 4th to 5th postoperative day the skin of the adrenalectomized animals developed a bluish color which was due to the growth of hair follicles with much melanin. Abundant and glossy black hair appeared externally on the 15 surviving adrenalectomized animals from the 34th to 38th days of life. and gray hair appeared on 6 of the controls from the 45th to 55th days while practically no hair grew on the other 4 rats.

When 10 rats which had been fed the deficient diet and on which the hair appeared externally from the 45th to the 50th days of life reached the age of 70 days, the hair was removed from their backs. Eight of these

1000

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